Conservation Genetics of the Java sparrow (*Padda oryzivora*) and an analysis of its viability

Thesis submitted by

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James Cook University

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ABSTRACT

The problem of how to conserve small and declining populations is currently receiving considerable attention in the ecological literature, particularly through the theoretical development of conservation biology and its application to endangered species conservation. This is true despite basic information on the natural history of most endangered species being very limited; not only for species that are very rare, elusive or living in remote areas, but also for species which occupy more accessible habitat (i.e. cultivated areas), such as the Java sparrow (*Padda oryzivora*).

In the research outlined in this thesis, field studies and molecular analyses were combined to establish the current population status, level of continued threat, contemporary connectivity among remnant populations and the genetic diversity of the endangered Java sparrow. Field work included intensive censuses at 6 sites across Central and East Java during the 2004 and 2005 breeding seasons. To gain information about the current scale of trading and trafficking of Java sparrows, the numbers of birds for sale were surveyed at 7 bird markets, mostly situated close to the bird census sites. Further interviews with bird trappers established the level of trapping and distribution of trapping sites.

DNA was extracted from both fresh whole blood (field samples) and tissue samples (museum specimens) and analysed using two different molecular marker systems – part one and two of the mtDNA control region and 5 independent nuclear microsatellite loci. MtDNA sequence data were used to infer phylogeography and historical demography of the Java sparrow, while, multi-locus microsatellite genotyping was used to assess contemporary connectivity and levels of genetic variation. In order to predict the future fate of the Java sparrow, a PVA and sensitivity analysis was also undertaken. Stochastic modelling was carried out using the program VORTEX.

The results of this study highlight that remnant populations of Java sparrow in Central and East Java are currently small and highly fragmented. Based on roost counts at 6 sites, population estimates range from 5.8 (± 0.2 SE) to 125.2 (± 1.7 SE). The total estimated population for Central and East Java did not exceed 1000

individuals. If other regions of Indonesia within the species' natural range have similar abundances, then the total Java sparrow population in Indonesia is likely to be at the lower end of the range of 2500 – 10,000 individuals that is currently used to classify the species as 'Vulnerable'.

The major threat from trapping and trading is still ongoing at a high level, with an average 59.3 % of the total population estimates being trapped during the study period. This threat is particularly severe in central Java, where market demands were mainly supplied by local wild caught birds. In contrast, in the east Java bird markets, introduced and captive bred birds were substituted to satisfy high market demand. These findings emphasize the potential abundance of Java sparrows that may occur in currently unstudied introduced populations on outer islands (e.g. Kalimantan), and the important role that introduced and captive bred birds currently have in mitigating further declines. They also highlight the potential usefulness of these introduced populations as part of future conservation schemes for the Java sparrow.

Analysis of mtDNA sequence data was used to infer the effects of historic habitat changes on population demography and genetic diversity in the Java sparrow. Despite an expectation that this species would have increased habitat availability during glacial maxima, analysis based on standard mtDNA mutation rates revealed that major climatic shifts have caused bottlenecking in Java sparrows similar to those observed in temperate species impacted by expanding ice sheets. Alternatively, using more recently derived and contrasting mtDNA mutation rates suggests the Java sparrow was bottlenecked during the expansion of rainforest in the early Holocene, and likely expanded during deforestation associated with the arrival of cultivation to Java. If correct, this finding adds to an increasing number of studies highlighting the impact of human colonization on the distribution and abundance of endemic species.

Microsatellite genotyping demonstrated that genetic variation in remnant Java sparrow populations was low, in the middle range of genetic variation observed for other endangered species. Levels of diversity among contemporary populations did not differ from historic samples. In addition, significant structuring was found among remnant but not historic populations, implying recent fragmentation and limited current inter-population movement. Therefore, it is likely that while recent

population declines have, as yet, had limited impact on genetic diversity, they have had a significant impact on levels of interpopulation gene flow.

Stochastic PVA modelling suggested that, under a best case scenario, Java sparrows would be able to recover. Sensitivity analysis revealed that the PVA models were most sensitive to mortality and fecundity schedules. However, the results highlighted that further field studies of these parameters are necessary to gain a more realistic assessment of the potential fate of the Java sparrow over both the short and longer term. PVA also suggested that if the current level of trapping continues Java sparrow will become extinct within a very short period of time. Given that terminating trapping seems an unlikely short-term management option, these findings highlight the immediate need to formulate a trapping/harvesting strategy that minimizes the risk of extinction.

The implications of the results of this research are as follows:

1. It is proposed that the conservation status of Java sparrow to be transferred from Vulnerable to Endangered (A2a,b,d; E). This research also provides a more robust, high quality data set that can be used for conservation status assessment.
2. Trapping remains the main threatening process and must be reduced before other conservation measures can be effective. The use of captive bred and/or introduced birds should be encouraged to meet market demands. Further population studies on introduced populations (e.g. in Kalimantan) are a necessity to develop the sustainable use of these resources.
3. For management purposes the Java sparrow can be considered as a single Management Unit. However, to develop a sound conservation strategy for this species, it is important to take into account the concept of "ecological exchangeability". For this purpose we need studies of behaviour, life history, and morphology relative to environment. Such studies will allow more meaningful assessment of biologically relevant differentiation among the remnant populations of the Java sparrow.
4. There is also a need for further research on demographic parameters and breeding biology to gain more realistic predictions of population viability.
5. There is a critical short-term need to formulate a trapping/harvesting strategy to minimize the extinction risk. Working thresholds need to be established as a

short-term management priority and as a basis for more effective and sustainable management strategies over the longer term.

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CHAPTER 1. INTRODUCTION

1.1. Background to the study

The problem of how to conserve small and declining populations is currently receiving considerable attention in the ecological literature, particularly through the theoretical development of conservation biology and its application to endangered species conservation. However, undermining these developments is the fact that even basic information on the biology-ecology of most endangered species is extremely limited. For example, reviewing BirdLife International's database (2006) revealed that the quality of data currently being used to classify most endangered bird species is *poor*. This observation is valid not only for endangered species that are rare, elusive, or living in remote areas, but also for species which occur in more accessible habitat (i.e. cultivated areas), such as the Java sparrow (*Padda oryzivora*).

The Java sparrow is a bird species endemic to the Indonesian islands of Java and Bali (MacKinnon and Phillipps 1993; Balen 1997), that has also been introduced widely from South-east Asia to other areas, such as the Christmas, Cocos-Keeling and Hawaiian Islands (Long 1981; Islam 1997). The species was formerly common in cultivated areas in Java and Bali, but over the last three decades populations have declined dramatically and become highly fragmented because of trapping for aviculture (MacKinnon and Phillipps 1993; Balen 1997). As a consequence, the Java sparrow has been listed as Vulnerable (A2b,d; A3b,d; C1) within its natural range (IUCN 2006). This assessment is based on population size and trend criteria derived from *poor* quality data detailed in BirdLife International (2006). More recent studies have been undertaken to assess whether population declines are continuing. Unfortunately, these studies applied rapid assessment methods that did not include replicate counts at each site (Laudisensius *et al.* 2000; Muchtar and Nurwatha 2001). Therefore, despite the baseline data obtained, they provide no associated estimate of error and the current demographic trends for Java sparrow populations throughout Java and Bali are unknown.

As well as the demographic problems associated with small populations sizes faced by a species such as the Java sparrow, very little is known about the genetic

consequences of population declines and fragmentation at this scale. Theoretically, population declines such as this increase extinction risk due to both the loss of genetic variation and associated increases in the level of inbreeding. For this reason, a common goal of many endangered species conservation programs is the maintenance of genetic variation. However, such goals are often set without a clear understanding of either the genetic/evolutionary history, or future potential trajectory of an endangered species.

Understanding or assessing how to best manage the genetic resources of a species both within and among small fragmented populations, while simultaneously maintaining demographic viability, is a complex task that requires the integration of a number of quite distinct but complementary data sets. Data are required on current demography and trends, contemporary connectivity as well as historical demographics, threats, behaviour, morphology, physiology, and biotic interaction. At present few comprehensive empirical data sets exist that can be used to develop and test either species-specific management options or general models of integrated management.

1.2. Aims

Based on the background above, the aims of this study were to:

1. assess the current population status,
2. assess the level of threat,
3. assess the historical demographic processes,
4. assess the connectivity among the remnants populations and the genetic diversity,
5. analyse the population viability,

for Java sparrow throughout its natural range in Java and Bali

Work undertaken in this project will allow the development of a comprehensive integrated management program to be tested in ongoing research, as well as to develop general models of how data sets can be integrated to develop such management options.

1.3. Overview of the study

Given the limited reliability of existing data on population sizes and demographic trends for the Java sparrow, I first conducted an extensive survey followed by an intensive census to assess the current distribution and abundance of remnant populations of Java sparrow in Central and East Java (Chapter 2). The following chapters (Chapter 3 and 4) then build up an appropriate research method for assessing genetic variation and population genetic structuring of the Java sparrow. Chapter 3 highlights the use of mitochondrial DNA (mtDNA) data to reveal historical demographic and evolutionary processes. Chapter 4 describes the use of multi-loci microsatellite genotyping to establish the effects of population declines and fragmentation on genetic variation and recent connectivity among the remnant populations. Chapter 5 reports the main finding on current threats to the Java sparrow, particularly the threat it faces from the aviculture and wild-bird trade. This chapter also describes the potential threat from avian malaria, which was assayed using PCR based analysis. Based on these findings and other published data, population modelling was then used to simulate future demography and to assess the population viability of the Java sparrow (Chapter 6). Chapter 7 integrates the findings from previous chapters and provides further detailed discussion of management alternatives along with implications for the conservation of the Java sparrow. Future research directions and needs are also discussed.

CHAPTER 2. CURRENT POPULATION AND DISTRIBUTION

2.1. Introduction

The historical data available on the Java sparrow within its native range is largely limited to distribution and habitat records (*Appendix 1*). In the early 1990s, van Balen (1997) comprehensively assessed existing locality records for the Java sparrow in Java and Bali. BirdLife International (2001) extended this assessment and further included the species' non-native range in Indonesia. To supplement these data more in-depth studies have been done since 1998: YPAL (*Yayasan Pribumi Alam Lestari*) surveyed the Java sparrow distribution in Java and Bali (Muchtar and Nurwatha 2001), and at a finer scale field surveys were carried out in Magelang (Anonymous 2003), Yogyakarta (Laudisensius *et al.* 2000) and Bali (Surata 2000). As expected these studies found that the remaining populations were small and highly fragmented (*Appendix 1 & 2*). However, most of these studies applied rapid assessment methods with no repeated counts at each site. Therefore, despite the baseline data obtained, they provide no associated estimate of error.

I undertook preliminary surveys in mid 2003 that aimed to locate populations for use in further detailed ecological and demographic studies. These surveys found that most previously identified populations no longer persisted at sites where they were originally observed (*pers. obs.* 2003; Nurwatha, *pers. comm*. 2003; Surata, *pers. comm.* 2003). These findings severely undermine the potential validity of published status and distributional data for this species and strongly suggest that further rapid decline may have occurred. This, combined with the fact that the Java sparrow continues to be in high demand on the Indonesian bird market, one of the main causes of their original decline, means that updating our knowledge of remnant populations using reliable methods is an essential and important conservation priority for this species.

2.2. Aims

This chapter reports the findings of detailed population surveys in Central and East Java during the 2004 and 2005 breeding seasons. The population surveys aim to establish the current distribution and abundance of the remnant Java sparrow populations, in particular in Central and East Java. Additional life history information was also obtained for each population. These data included breeding status, phenology and nesting site locations/characteristics.

2.3. Methods

2.3.1. Preliminary surveys

During May – July 2003, preliminary (presence/absence) surveys were undertaken at all roosting or nesting sites in Central and East Java where the Java sparrow had previously been recorded. At each site surveys for the presence of Java sparrow were done once or twice per day, in the morning (6:00 - 9:00) and/or afternoon (15:00 – 18:00). Surveys were repeated two to three times at each site. In addition, interviews with local people, temples guard officers and local birdwatchers were conducted to obtain secondary data on the persistence of the Java sparrow at each location during the previous one to two years. Moreover, bird market surveys in Yogyakarta, Surabaya and Malang, and further interviews with bird trappers were used to identify other potential remnant populations that had not been recorded previously. Nine potential new sites were identified from these sources and included in the presence/absence surveys. Following these initial surveys, detailed population censuses were carried out at all sites where the presence of the Java sparrow was confirmed. In addition, data on breeding status, phenology and nest site selection were obtained where possible.

2.3.2. Population estimate

In order to estimate remnant population sizes, detailed censuses were carried out during the 2004 and 2005 breeding seasons. Where possible two independent census methods were applied per location: direct counts at staging sites, and mark-

recapture methods. In general, direct counts were carried out as each population of birds left their staging site and moved to roosting sites at approximately the time of *adzan Maghrib* (the Moslem call to evening praying), within a half hour of dusk each day. Census methodology varied slightly between locations. These variations are outlined in the results section.

Mark-recapture methods were impracticable at some sites and so this method was only applied in Kepurun, Prambanan and Malang. The capture techniques used were mist-netting (Malang) and double-clap trapping (Prambanan and Kepurun). The mist-net was set up using 5 m metal poles on the roof top of the third floor of a Malang regency office (ca. 20 m) across the route of birds returning to a roosting tree (*Hymenaea courbaril,* ca. 27 m height). The double-clap trap was set up on the ground around a paddy area where the birds were expected to land for feeding. Live decoy birds were used to attract flocks into the trap area. Prior to release, all trapped birds were banded for individual recognition using combinations of colour rings.

Population estimates from the direct counts were derived using descriptive statistics (mean and standard error from replicated counts). To estimate the relative abundance at each site, independent population estimates were obtained during the breeding (December – August) and post-breeding (September – November) Seasons. The length of the breeding season was based on data we obtained from Java sparrow populations in Pambanan and Malang during this study, and differs slightly from previous reports (review in Balen 1997; BirdLifeInternational 2001). Since the recapture rate was very low, with no recaptures occurring in Malang and Kepurun, mark-recapture population estimates were made using the resight method with the EcoMath program (Krebs 1999). Assumptions associated with this methodology are that each population is closed, that there is equal probability of capture among marked and unmarked individuals and that sampling is random (Bibby *et al.* 2000).

2.3.3. Breeding status, phenology and nest sites

Nest site characteristics were documented for all known nests. Unfortunately, detailed data on reproductive output per nest could not be obtained, as most nests were inaccessible. Therefore, breeding cycle assessments for each nest were carried out by linking behavioural observations of adults and juveniles to breeding cycles in

captive birds. The behavioural clues used related to aspects of nest-building and courtship activity, nest occupancy periods, and the occurrence of young birds. These were compared to the published breeding cycle data as follows: incubation period 13-14 days, nestling period ~21 days (Restall 1996). Fledglings differ from adults in having a pale dull brown and grey plumage, and dark beak for the first 4-5 months after fledging (Restall 1996; Salem 2005).

2.3.4. Sex determination

Java sparrow sexes have monomorphic plumage, so it is hard to determine an individual's sex without having the bird in hand. However, males usually have more massive bills than females. In the breeding period, male and female birds are also slightly different. Male birds have brighter red eyelid colour and the bases of their beaks are more swollen. Molecular sexing was applied to determine the sex of the chicks (see below).

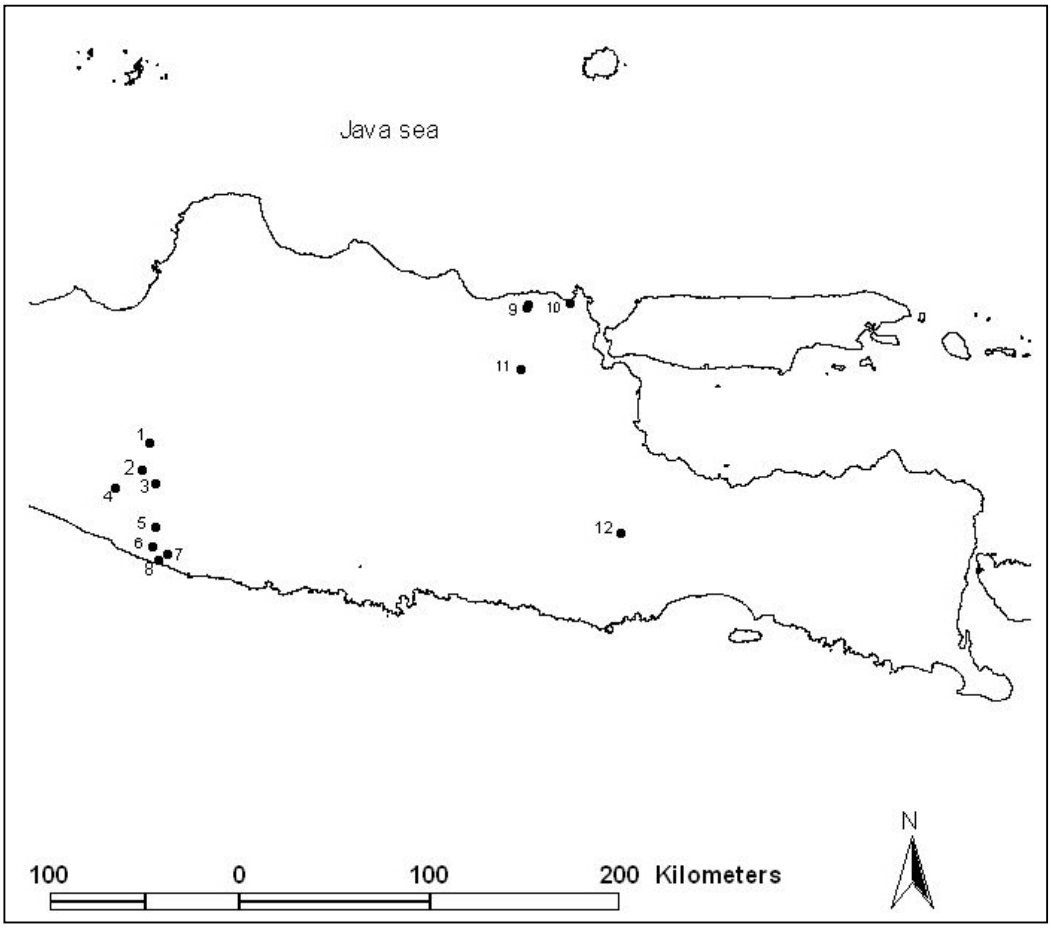
Molecular sexing followed the protocol developed by Fridolfsson and Ellegren (1999). This method makes use of the presence and absence of a sex-dependent DNA fragment, e.g. W chromosome in birds. PCR produces two different size products in females and one in males. 25 ul PCR reactions consisted of 10-20 ng of DNA; 10x PCR Buffer (200mM Tris-HCl (pH 8.4); 500mM KCl); 2.0mM MgCl2; 5 pmol of each primer; 0.15 mM of each dATP, dTTP, dCTTP, dGTP and 1 unit of Tag polymerase (Life Technology). PCRs were run using the following cycle conditions: 94C for 90 sec; 30 cycles for 45s at 50C, 30s at 72C, 30s at 94C; and 60s 50C, and 5 m at 72C. The primers used were 2550F (5'-GTTACTGATTCGTCTACGAGA – 3') and 2718R (5'- ATTGAAATGATCCAGT GCTTG -3').

The sex ratios in each Java sparrow population were determined using both the adult and fledgling birds that were caught during trapping. Chi-square goodness of fit (X^2) was used to test whether the observed sex ratios deviated from the expected sex ratio of 1: 1.

2.4. Results

2.4.1. Preliminary surveys

During the preliminary (presence/absence) surveys across Central and East Java in 2003, Prambanan (Yogyakarta) and Malang (*site 61 & 75, Appendix 1*) were confirmed as the only previously identified nesting locations where birds were still regularly observed. Bird market surveys in Yogyakarta/Gunungkidul, Surabaya and Malang, and further interviews with bird trappers indicated other potential remnant populations existed that had not been recorded previously. Nine potential new sites were identified from these sources; however at only four of these sites was the presence of Java sparrow confirmed (Figure 1).



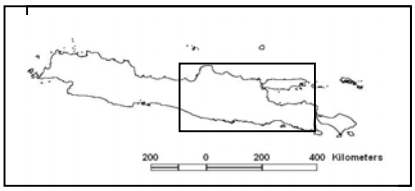


Figure 1. The distribution of study sites: 1. Magelang, 2. Kepurun, 3. Prambanan, 4. Purosani, 5. Gua Maria Tritis, 6. Gua Mandung, 7. Gupakwarak,, 8, Jhotak, 9. Sugio, Lumajang, 10. Ujung Pangkah, 11. Dadapan, Babat, and 12. Malang

2.4.2. Present study

2.4.2.1. Population estimates

A total of nine extant populations were identified. These were located in village/rice field areas (Magelang, Kepurun), a temple complex/rice field (Prambanan), woodlands on limestone hills (Gunungkidul: three populations, Gresik), and in an urban area (Malang, Purosani), all at between 200 - 265 m a.s.l. Population estimates at each site based on roost counts ranged between 4.5 (± 1.21) and 126.3 (± 3.16) individuals (Figure 2), while those based on mark-recapture data ranged between 70 (44 - 112) and 259 (148 – 452). Where estimates were obtained using both methods, roost counts provided consistently smaller numbers per site (Figure 3), on average 0.22 of the other count result. The total maximum population estimate of Java sparrows across all sampling sites in Central and East Java did not exceed 1000 birds. Details for estimates at each site are described below (Section 2.4.2.2).

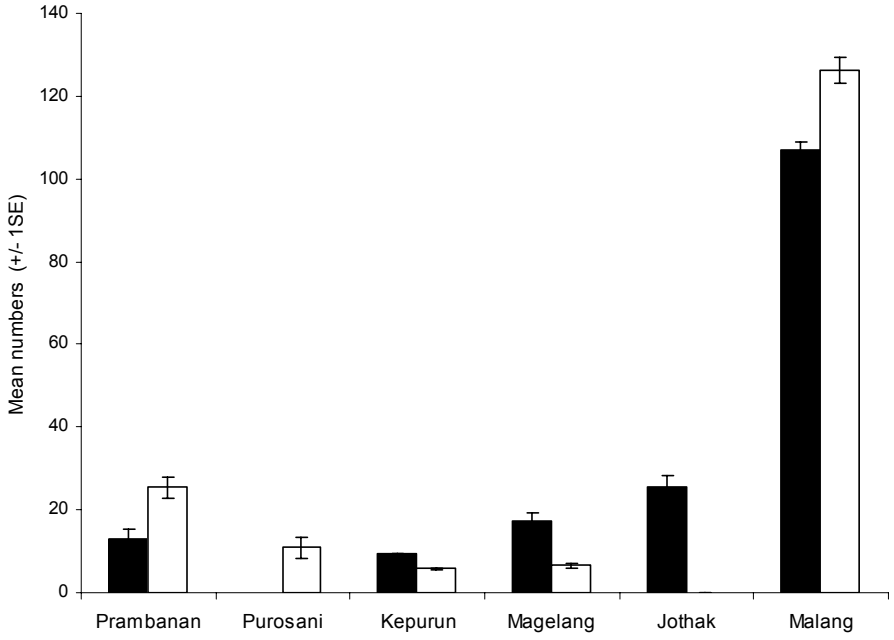


Figure 2. Population estimates of at each study sites based on roosting counts. (Black bar: 2004 counts; white bar: 2005 counts)

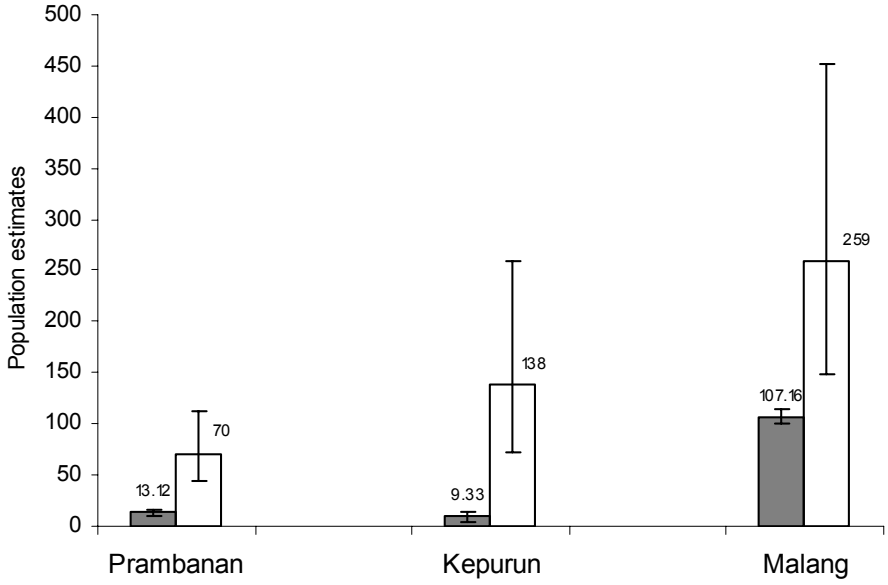


Figure 3. The estimated numbers of the Java sparrow obtained using two different methods: direct roosting counts (grey bar; ± 2SE) and mark-recapture (white bar; ± CL 95%)

2.4.2.2. Site-specific accounts

a. Prambanan

The study area is situated in the largest temple compound dedicated to Shiva in Indonesia. At the site there are three main temples: Siwa, Brahma, and Wisnu, and three smaller temples, Nandi, Angsa and Garuda. The height of these stone temples ranges between 24.4 m (Angsa) and 46.5 m (Siwa). The Java sparrow used the temples as nesting and roosting sites, particularly Siwa and Brahma (Aji 1999; Laudisensius *et al.* 2000). During this study restoration was being undertaken at Siwa which caused birds to move to, and utilise, other temples.

Direct counts were implemented monthly in November and December 2004 or two-weekly from May to September 2004 and from January to December 2005. The counts were made when the birds moved from a Casuarina (*Casuarina junghuhniana*) 'staging roost' tree to a kapok (*Ceiba pentandra*) roost tree, ca. 100m south-east of the nesting sites in the temples. Assuming that all the Java sparrow nesting in the temples were using the kapok tree as their night roost, the number of nesting birds observed suggested that there could be birds nesting in other sites outside the temples complex that also used the roost tree. The average number of birds estimated from direct counts in the Prambanan temples complex during each breeding season were 13.12 (± 1.23, n =8) in 2004 and 25.4 (± 2.68, n = 8) in 2005.

Twenty birds were marked and released from April to August 2004. Only one marked bird was re-caught. However, during subsequent observations from August to December 2004 in the temple complex, nine marked and 156 unmarked birds were sighted. The frequency of resighting of specific marked individuals ranged from 1 to 8. Using the resight estimation formula (Krebs 1999), the total Java sparrow population within the temple complex was estimated at 70, with 95% confidence limits (CL) from 44 to 112.

b. Purosani

Purosani is located at the centre of Yogyakarta city. The Java sparrow was encountered under the roof of the seventh floor of the hotel Melia Purosani. The first encounter was reported by a local birdwatcher (Lim, *pers comm*.) in December 2004, when he observed 6 birds. Since then, I conducted five counts during March - April 2005 and a further 5 counts during November - December 2005. The total average

number of Java sparrow encountered was 11.66 ± 1.11 (n=6). I also recorded two juvenile birds in April 2005.

The Java sparrow used a gap beneath the eaves of the hotel for nesting. There are some Casuarina trees (*Cassuarina junghuniana*) growing around 5 meters from the nest sites. The trees are a suspected 'staging roost' used by birds en route to other roost sites. From this "staging roost' birds disperse to elsewhere across the city. To date, I have not located the night roosting sites of this population.

c. Kepurun

Kepurun is a typical village in a rural area of Java, located in the south-eastern foothills of Mt Merapi. At this site the Java sparrow were observed in rice fields with *Albizzia falcataria* trees growing on dikes (*galengan*) surrounding the fields. Birds appeared to use these trees as both roosting and nesting sites. However, only one nesting tree could be clearly identified at the site. In this tree birds were observed nesting in old woodpecker holes.

A census was conducted using the encounter rate method. I walked along the dikes and counted all birds encountered during 2 hours in the morning (7.00- 9.00) or in the afternoon (15.30-17.30). Censuses were undertaken in July, November and December 2004, and in February and March 2005. During 2004, maximum estimates of 15 birds with an average of 9.3 ± 2.84 (95%) per survey were obtained. In 2005 only six birds were encountered during one survey.

Thirty birds were marked and released from July to September 2004. Only three birds were resighted since then. Using a resight estimation model (Krebs 1999), the total Java sparrow population estimate for this site was 138 (73 – 259; CL=95%).

d. Magelang

The roost and nest sites of the Java sparrow at this location were situated at the 'Istiqomah' mosque in the Panca Arga military residential complex. The mosque is a typical small Indonesian mosque, a pyramidal-roof building with a dome on its top. The total height of the mosque is around 15 m. The plants growing in the mosque's yard are Pine (*Pinus merkusii*), and Turi (*Sesbania grandiflora*). Paddy fields are found near the residential complex.

At this site direct counts were carried out at two-week intervals from July to December 2004, and monthly from January to December 2005. The total population during the 2004 breeding season averaged 17.25±1.25 (n = 4), while during the 2005 breeding season it averaged 6.58±0.65 (n = 12). During this study two active nests that were established on plastic lining inside the roof of the mosque were found on August 2004. The first nest contained three new hatchlings and the other four hatchlings and three eggs. Nests and unhatched eggs of Eurasian tree sparrow were also found in the ceiling. Also, in the rafters of the ceiling were found two dead Java sparrow chicks and eggshell. In September 2005 the mosque officers "harvested" 22 chicks from four different nests. Fortunately, in December 2005, they agreed to release the birds following blood sampling and ringing by us. However, up until February 2006, none of the released birds were resighted in the mosques.

e. Gunungkidul karst

This area is the western part (60%) of the Gunung Sewu karst region. It covers more than 1300 km^2 and comprises over 10,000 cone hills (Haryono and Day 2004). Since the 1970s this area has been known as the principal region supplying the Java sparrow to the bird markets in Yogyakarta and surroundings (*pers. obs*.). The use of *Gelatik* (= Java sparrow) in the names of two sites in Gunungkidul, namely *Pulau Gelatik* (Gelatik Island) and *Gua Gelatik* (Gelatik Cave), likely highlights the previous abundance of the birds in this region.

However, during a previous study in 1999, only a small number of birds were found across eight localities in this karst area (Appendix 2) (Laudisensius *et al.* 2000). In 2003, a second survey by us failed to confirm the presence of the Java sparrow at these locations. However, four new sites were identified: Jothak, Gupak Warak, Gua Maria Tritis and Gua Mandung. During the 2003 survey I encountered 20, 13, 5 and 7 Java sparrow at these locations respectively. Unfortunately, during the follow-up intensive census period from September 2004 to February 2005, Jothak was the only site where the Java sparrow was consistently encountered. I obtained further bird sightings only once at Gua Maria, 3 birds in September; twice at Gua Mandung, 7 and 3 birds in September and February respectively, and not at all at Gupak Warak. Since the distance between Gua Maria and Gua Mandung is only 1.7 km, I considered the Java sparrow present in these two sites as one population.

Using roost counts the breeding population of the Java sparrow at Jhotak was estimated at 25.66 (± 4.25, n= 3) with a maximum of 34 birds. The post-breeding population was estimated at 31.5 (± 2.5, n= 2) with a maximum of 34 birds. At this site the birds used crevices in the wall of a sink-hole cave (*luweng*) as nesting sites and occupied *Lantana camara* bushes at the edge of the top of the cave entrance as a 'staging' roosting site. To date, I have not located the night roosting site(s) of this population.

f. Malang

This site is the Malang county office complex, which consists of seven 2-4 storey buildings. Adult and juvenile birds were observed to roost on buildings and/or in tanjung (*Mimusops elengi*) and flamboyan (*Delonix regia*) trees in this area. These trees appeared to serve as 'staging' trees to the night roosting in a *Hymenaea courbari* tree.

Direct counts were carried out as birds moved between the staging and night roosts at 17:20 to 17:45 each night during the *adzan Maghrib* (the Moslem call to evening prayer). Similar counts were performed once or twice per month from February 2004 to December 2005. The total average number of birds encountered during the 2004 breeding season was 107.16 (± 3.76, n=12); and in the 2005 breeding period the average was 126 (± 3.16, n=13). During the day I also found adults and juveniles roosting and/or nesting in the tanjung tree and under the roof of the fourth floor of the main building. The number of birds encountered during the day was less than the afternoon counts, with the maximum being 36 birds.

Twenty-four birds were marked and released from March to July 2004. However, none of the marked birds were re-caught. During subsequent observations from August to December 2004, 9 marked and 167 unmarked birds were sighted. The resighting frequency for individual marked birds range from 1 to 3. Using the resight estimation formula (Krebs 1999), the total Java sparrow population at Malang was estimated at 259 (44 – 112; CL 95%).

g. Lumajang and Gresik

Information obtained from bird trappers suggested that Dadapan was the most recent site where they had caught birds. However, three surveys at this site in

October and December 2004 and March 2005 did not encounter any Java sparrow. This was also true for Sugio which was surveyed four times from October 2004 to March 2005. Meanwhile, at Ujung Pangkah, and Gresik, 4 of 8 surveys encountered the Java sparrow. One bird was observed in both October 2004 and March 2005 and six birds in April and June 2005. I suspected that these birds used limestone caves in the region as nesting sites.

2.4.2.3. Breeding

Table 1 provides observed and/or estimated breeding periods for the Java sparrow at each study site. Previous reviews found that within its natural range the Java sparrow breeds during extended periods from February to August (BirdLifeInternational 2001). However, Table 2.4 shows that at the present study sites breeding extends for even longer periods, particularly in Prambanan and Malang, where breeding birds were observed in all months except September to November. In Gunungkidul breeding occurs during the periods when either rice paddies (February – April) or *gaplek* cassava (August) are harvested. At other sites breeding is also sporadic and so may be related to the abundance of locally available resources.

The nest sites used by individual Java sparrow seemed to encompass almost any protected nesting hollow and varied according to location. They included gaps beneath the eaves of buildings (Malang, Purosani and Magelang), under a parabola antenna (Malang), holes among the stone slots of the Prambanan temples, crevices in limestone caves (Gunungkidul and Gresik) and nesting cavities in trees (Prambanan and Kepurun). Java sparrows were observed occupying barbet's nest holes in Randu Alas (*Gossampinus sp.*) in Prambanan and attacking a woodpecker in a fight for a nest hole in an albizia tree (Sumiyar, *pers.com.*, 2004) in Kepurun. In general, nests were of an untidy and loose construction with the material used varying from fresh and dried grass, to plastic rope, and small plastic sheets from cigarette boxes.

During this study only 4 clutches were observed, two in Prambanan and two in Magelang. Clutches ranged from 4 to 6 eggs (Prambanan), and 4 to 7 eggs (Magelang), with an average of 5.5±0.91 (n=4). However, the number of fledglings encountered per nest was generally smaller, ranging from 2 to 6 birds (Malang). I observed a group of 12 juveniles associated with a single paired adult in Malang, but

it is likely these juveniles came from different clutches. During this study I also recorded three and four dead nestlings, at Magelang and Pambanan respectively. All were below nest sites, suggesting that they had fallen for unknown reasons.

Table 1. Breeding time of the Java sparrow in the study sites

Site	Month											
	Jan.	Feb.	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Prambanan	■	■	■	■	■	■	■	■				■
Purosani		■	■	■								
Kepurun					■							
Gunungkidul		■	■	■				■				
Magelang				■		■		■		■		
Malang	■	■	■	■	■	■	■	■				■
Sumbawa*											■	
Madura*			■				■	■				
Dadapan*			■									
Kalimantan*										■	■	

Note: * base on the finding of young birds in bird markets in Surabaya

2.4.2.3. Sex ratio

Given that the sex of the birds could not be assessed at a distance, e.g. during bird counts, the sexes of the Java sparrow were assessed using wild-catch birds and samples collected from the bird markets. A total of 66 adult birds were sexed, 29 females and 37 males. Molecular sexing of 20 juvenile birds resulted in 12 females and 8 males. Sex ratios for either adult or juvenile Java sparrow did not deviate from 1:1 (X^2 = 0.97; P= 0.32 and X^2 = 0.80; P= 0.37, respectively).

2.5. Discussion

2.5.1. Comparison to pre-2004 surveys

While previous studies were primarily designed to identify the distribution of the Java sparrow rather than to estimate abundance, population estimates were reported. As expected, most of these studies appeared to produce underestimates of population sizes, due to poor or inappropriate counting methods. Muchtar and Nurwantha (2001) reported only 109 birds at 63 sites during their study across the islands of Java and Bali, while in the Yogyakarta region during a January-February

survey in 1999, Laudensius *et al.* (2000) found 125 Java sparrows in 21 sites. A better population estimate was reported for Prambanan and Magelang populations (Anonymous 2003), in which replicated counts were conducted at nesting and/or roosting sites.

My current survey covered about half the region examined during previous studies (Aji 1999; Imansyah *et al.* 1999; Laudisensius *et al.* 2000; Muchtar and Nurwatha 2001; Anonymous 2003). However, the survey program was more intensive and applied more robust counting methods. For this reason, it is expected that the estimates obtained are more reliable and are appropriate for identifying the recent status of the Java sparrow.

In total, the presence of Java sparrows was confirmed at 10 sites in this study, compared to 24 sites in previous studies (Laudisensius *et al.* 2000; Muchtar and Nurwatha 2001). In central Java only 2 of 21 previously occupied sites were still extant, but 6 new sites were located. Meanwhile in east Java only 2 occupied sites were recorded, compared to 3 known previously. One new site, however, was confirmed. I also found that Java sparrows were no longer present in other temple precincts in Yogyakarta, where nesting had previously been reported,. Temples at these sites did not appear to have holes or gaps among the stone appropriate for nesting. Interviews with temple guardian staff supported this observation.

This study found that the Java sparrow still occupied nesting and/or roosting sites at Prambanan and Malang, but not feeding sites at these locations. However, two new feeding sites were observed at Kepurun and Gresik. This finding suggests that the presence of Java sparrows at specific feeding sites may be short-lived or intermittent. There are three possible explanations for this phenomenon. Firstly, Java sparrows may move over wide areas with their presence in a specific location only corresponding to a short term local abundance of food resources (Restall 1996). Secondly, it is possible that birds are always present at each site, but that small population sizes make it difficult to consistently observe them with current sampling methods. Thirdly, populations may become locally extinct because of high intensity trapping in these feeding sites (see below).

Except for the population at Purosani, all new positive location records resulted from information gathered from trappers. These sites were considered ‘traditional’ trapping sites, suggesting that the birds have been in these locations for

long periods of time. All trappers interviewed complained about the increasing rarity of Java sparrows over the last 10 years, suggesting a decline at trapping sites over this period. The Purosani nesting site occurs at a relatively new hotel, built in 1994. It is likely that this was a relatively new nesting colony that has moved from another site or originated from dispersing immatures.

2.5.2. Population estimate

Total population estimates of the Java sparrow in Central and East Java using point counts ranged from 137 to 209 (2004) and from 121 to 204 (2005). Using mark-recapture estimates for three sites resulted in population estimates ranging from 299 to 889 (2004). This gives a total population of Java sparrows within the surveyed area of no greater than ~1000 individuals. I believe that this result is the best estimate of the number and distribution of remnant Java sparrow populations obtained to date. If this pattern is consistent across other regions, then Java sparrow populations within the species natural range remain very small and fragmented. However, our findings also imply that other small populations may exist that are still to be found if similar intensive surveys are undertaken in other areas. More recently, other new locality records have been reported in the area surrounding Yogyakarta (e.g. Imogiri and Keraton; Kurniandaru & Wardani, *pers. comm* 2006) and in Semarang and Purwodadi (Sigid, *pers.comm,* 2006). For this reason, I believe that the total Java sparrow population in Indonesia is likely to be at the lower end of the range from 2500 – 10000 individuals, that is currently used to classify the species as 'Vulnerable' (BirdLifeInternational 2006).

Each count method used in this study resulted in substantially different estimates. Here, I briefly discuss the advantages and disadvantages of each method. For direct roost counts, locating Java sparrow roosting and/or nesting sites is the key factor in estimating population size. This study found that there was a staging site/tree associated with each nesting site. In addition the roosting site may or may not be close to the staging site. Birds left the staging site to roost at approximately the time of Adzan Maghrib, within a half hour of dusk each day. Therefore counting the birds at this time and in this location produces a consistent and repeatable

comparative estimate of the local population. Failure to locate all staging sites, however, will produce underestimate of population size for the studied areas.

In addition there were potential sources of bias in this method. Firstly, low light intensity at dusk may produce observer error and secondly, the occurrence of other species in the flying-flocks. This phenomenon was a particular problem in relatively big colonies. In Malang I found small mix-species flocks that included Oriental White-eye (*Zosterops palbebrosa*). Other finch species (*Lonchura punctulata*) were also found in the staging trees in Prambanan and Malang, but these species were not mixed with Java sparrow.

Mark-recapture provided much larger population estimates, ranging from 2 to 10 times greater than the roosting/point counts, but this method also produced much wider confidence limits. This was because of the use of Bowden's resight estimator which does not apply restrictive assumptions regarding the equal sightability of the sampled birds (Krebs 1999). In addition to more variable estimates, this method also required significantly more time and resources. For the latter reason, even though estimates using mark recapture suggest that roost counts produce consistent and significant underestimates of the total population at any given site, we consider roost counts to be the preferred census method for any future population monitoring program for the Java sparrow. This is because, once appropriate roosting locations have been identified, roost counts allow cost effective and relatively precise comparative estimates to be obtained over time at individual locations. Such counts can then be scaled to absolute population estimates by combining them with intensive mark-recapture methods at appropriate intervals.

The findings of this study confirmed that remnant Java sparrow populations currently consist of very few individuals and that the distribution of these remnants is also highly fragmented. Therefore, levels of genetic diversity in the Java sparrow may also have deteriorated and become spatially structured across these remnants. This possibility is further examined in Chapter 4.

CHAPTER 3. INTRASPECIFIC PHYLOGEOGRAPHY

3.1. Introduction

Long-term global climate fluctuations are widely believed to be the major historical process influencing the genetic variation of natural populations (Hewitt 2000). Numerous studies of temperate birds across a range of habitat types provide evidence of low genetic diversity due to population bottlenecking in glacial refugia, followed by rapid post-glacial (recent) population expansions (e.g. Zink and Dittman 1993; Merila *et al.* 1997; Fry and Zink 1998; Pestano *et al.* 2000). Similar patterns are also evident among tropical seabirds (Peck and Congdon 2004) and some tropical forest species (Brook *et al.* 1997; Bowie and Fjeldsa 2004) not directly impacted by ice sheets. However, this is not the case for all tropical forest birds. For example, the phylogenetic history of the upland forest superspecies *Xiphorhynchus spixii*/*elegans* was created by interactions among geology, sea level changes, and hydrology (Aleixo 2004). A lack of appropriate studies means that virtually nothing is known of the potential impact of these same phenomena on tropical open woodland or savannah species.

During the last glacial maximum of the Pleistocene (about 18 ky BP), sea level was ~120 meters below present levels. In Southeast Asia this resulted in Sumatra, Borneo and Java being connected to the Asian mainland, forming Sundaland (Heaney 1991). The paleoclimate in Java at this time was cooler and drier, with these conditions resulting in the expansion of seasonal forest and savannah (Kazuko Urushibara-Yoshino 1997). However, about 18 - 9 ky BP (Whitten *et al.* 1997), or 5 – 10 ky BP (Kazuko Urushibara-Yoshino 1997), the climate became warm and wet, allowing rainforest to develop and expand (Kazuko Urushibara-Yoshino 1997). The consequences of this change for woodland and savannah species in Java are unknown.

3.2. Aims

Effective conservation planning for the Java sparrow requires information on current levels of population fragmentation and inter-colony gene flow, as well as on the possible genetic consequences of the rapid decline in numbers over the last 30 - 40 years. Before any of these more recent genetic processes can be interpreted in detail, it is necessary to have a clear understanding of the influence of historical processes on Java sparrow genetic diversity over geological time scales.

To this end, I used variation in mtDNA control region sequences to analyse intraspecific phylogeography for the Java sparrow. Understanding the phylogenetic history of this species not only provides valuable information that can be used to examine factors influencing current levels and patterns of genetic variation in this threatened species, but also allows us to establish and compare the potential influence of historic glacial cycles on this tropical woodland species to the known effects of these phenomena on other non-woodland taxa elsewhere in the world.

3.3. Methods

3.3.1. Population sampled

In total 104 DNA Java sparrow samples from throughout the species natural range, including Java (n=72), Madura (n=21), and Bali (n=11), were sequenced. An introduced population from Kalimantan (n=11) was also included in this study. Samples from wild populations were obtained using mist-nets or clap trap nets during population studies in 2004 and 2005 (Figure 2). Whole blood samples were collected from each individual by clipping toe nails. Blood samples were then stored in Queen's lyses buffer at 4^{o}C (Seutin *et al.* 1991). Mr. Sang Putu Kaler Surata kindly provided an additional 11 total DNA samples from birds caught in Bali. In addition, 29 foot-skin samples were obtained from historical museum specimens collected at 4 sites across Java during the period 1932-1941, prior to any perceived decline (Figure 4). For comparative purposes, samples were also obtained from an introduced population from the Cocos-Keeling Islands (Appendix 3).

3.3.2. DNA extraction

Whole blood and tissue from foot pads were used as starting material for DNA extractions. For blood samples, approximately 45 µl blood/Queen's Lysis sample was extracted with a standard phenol-chloroform extraction protocol (Bruford *et al.* 1998) or using the DNeasy® Tissue Kit (Qiagen Pty Ltd). In the first protocol, samples were digested with proteinase K (10-40 mg/mL) in an extraction buffer at 37˚C overnight. Purification of DNA was carried out with one extraction with phenol:chloroform:isoamyl alcohol (24:24:1) wash and one extraction with chloroform-isoamyl alcohol (24:1) wash. Precipitation of DNA was done with 2 volumes of absolute ethanol, followed by a washing step in 70% ethanol. DNA was then resuspended in TE buffer (10 mM Tris, 1mM EDTA, pH 7.2). Meanwhile, the second protocol followed the recommended protocol for animal blood (Qiagen Pty Ltd).

For tissue, approximately 1- 2 mg of footpad was ground using a micro-pestle in a 1.5 microlitre (µl) microfuge tube. DNA extraction was carried out with the DNeasy® Tissue Kit (Qiagen Pty Ltd), following feather extraction protocol. Specific care was taken with the museum samples in order to prevent cross-contamination with DNA obtained from blood samples. All museum specimens were extracted at a different time under a UV laminar flow unit, using a different set of reagents, pipettes and other equipment from those used for DNA extraction from blood samples.

The quality of DNA products was then validated with electrophoresis, by loading 5 µl of resuspended DNA along with 5 µl of x 1 TA buffer and 2 µl of loading dye (Bromophenol Blue) onto a 2 % agrose gel. Ethidium bromide (EtBr) was included in the gels to visualise the DNA. Gels were run in x1 TBE buffer at 45 MA for approximately 25 minutes. DNA was detected using ultraviolet light (GelDoc 1000 image system, BIORAD). The Hoefer® DyNa Quant® Fluorometer was used to measure the average quantity of DNA product from the above protocols.

3.3.3. Polymerase chain reaction and sequencing

The polymerase chain reaction (PCR) was used to amplify a 450 base fragment of the mtDNA control region, spanning parts of domain I and II. Initially, the primers FireC1F1 (5'- TTTTCCTHNTGACTTTTAGGGTATG –3') and FinchC1R1 (5' – GGGATGGTCCTGAAGTTACAAC – 3') (Sorenson and Payne 2001) were used, but amplified poorly. For this reason a species-specific internal forward primer GJ1F (5' – GGGTATGTACAAAATGCATCGCA – 3') was designed and paired with FinchC1R1. Because DNA extracts from museum specimens were of poorer quality, only a smaller portion of the same region could be sequenced that did not include approximately the first 70 bp. This was done using two sets of species-specific primers that amplified a 180 bp and 165 bp fragment respectively with an ~10 bp overlap: GJ2F (5' GGCACATTTTTGCTTCAGGT -3') and GJ2R (5'- TAACCAGGTCCTCTGGCTTG -3') for the first fragment and JS3F (5'- CTAGGGTTGGGTGCAGTAGAAAA – 3') and FinchC1R1 for the second.

Each PCR was carried out in a 25 µl reaction volume containing 10x PCR Buffer (10mM Tris-HCL (pH 8.4), 50mM KCl), 2.5mM $MgCl_2$, 20pmol of each primer, 0.8µg/µl of bovine serum albumin, 0.1 mM of each dATP, dTTP, dCTP, dGTP and 1 unit of *Taq* polymerase (Promega). Thermocycling conditions were as follows: initial denaturing steps of 95° C - 90s, 35 cycles denaturing at 95° C - 30s, annealing at 55° C - 60 s, extension at 72° C- 90s, and a final extension step of 72° C – 7m. The same cycling conditions were used for museum samples, except that they were repeated for 50 cycles. Double strand PCR products were purified by ethanol precipitation or spin column purification (Ultra Clean Tm, MO BIO Inc), prior to cycle sequenced using DYEnamic ET Dye Terminator Kit (MegaBACE). Sequencing products were purified and screened using MegaBACE™ DNA Analysis Systems at the Genetic Analysis Facility, James Cook University, Townsville.

3.3.4. Statistical analysis

3.3.4.1. Historical demography

Sequences were aligned and edited by eye using Proseq version. 2.9 (Filatov 2002). The evolutionary association between haplotypes was reconstructed using a median-joining network (Bandelt *et al.* 1999), performed in program Network 4.112

(www.fluxus-engineering.com). The tolerance level value (epsilon) was set to 0, to gain the smallest number of alternative nodes between haplotypes.

In order to assess the historical demography of the Java sparrow two different approaches were used. Firstly, I applied a mismatch distribution of pair-wise sequence differences among all individuals (Rogers and Harpending 1992). A unimodal mismatch distribution is expected in a population that has rapidly expanded following a significant bottleneck, while a multimodal distribution is expected for populations which are in equilibrium and have maintained a constant size over a long period of time (Slatkin and Hudson 1991; Rogers and Harpending 1992). These statistical tests were performed using Arlequin version 3.0 (Excoffier *et al.* 2005) and DnaSp version 4.00 (Rozas and Rozas 1999). The expansion coefficient (*S/d*), where *S* is the number of variable sites and *d* is the mean number of pairwise nucleotide differences, was also calculated to test for possible differences between recent and historical population size. A large value is a sign of recent population expansion and a small value indicates a population with relatively constant long-term population size (von Haeseler et al. 1996). Secondly, I calculated Tajima's *D* (Tajima 1989), and Fu's *Fs* (Fu 1997) statistics. Patterns of significance among these tests can distinguish different population processes, where significant negative values of both *D* and *Fs* indicate population expansions.

The mismatch distribution was also used to estimate the timing of any identified demographic expansion (Rogers and Harpending 1992) implemented in Arlequin. The generated tau (τ) value was converted into year before present (BP) using the equation $t = \tau / 2u$ (where $u = \mu k$; μ=mutation rate per site and year, k=sequence length), multiplied by the generation time (Rogers 1995). The corresponding confidence interval (95%) was calculated using bootstrapping. The mutation rate for the Java sparrow has not been previously estimated. Therefore, to obtain accurate approximation of the period during which any expansion may have occurred I used two different mutation rates. Firstly, I used a μ value of 14.8%, in accordance with the rate calculated for the same mtDNA control region Part I and II in greenfinch (Merila *et al.* 1997) and dunlin (Wenink *et al.* 1996). This is a standard value of μ used in numerous previous studies to calculate expansion coefficients of this type. It was used here to generate valid comparative estimates. Secondly, I used 71.2%, which was as an average substitution rate across parts I and II control region

derived from a μ value of 96% published for the HVRI control region of Adélie Penguins (*Pygoscelis adeliae*)(Lambert *et al.* 2002). This is the most rapid mutation rate currently documented in the literature and so it was used to produce the most recent possible estimate for any identified population expansion. The ratio of the number of substitutions in control region part I to those in control region part II is 2.07 for Fringilline finches (Marshall and Baker 1997). This translates to a substitution rate of 46.46% for control region part II and to an average of 71.2% for parts I and II combined, as used in my analyses. This calculation follows the same protocol used to calculate a combined μ value for parts I and II of the control region by (Wenink *et al.* 1996). Generation time was assumed to be one year, which is the age at first breeding of Java sparrows.

3.3.4.2. Genetic variation and population structure

To improve sample sizes and facilitate robust analyses, four populations were defined from the samples obtained according to their island of origin. These populations were: Java, Madura, Bali and Kalimantan. The number of haplotypes, number of polymorphic sites, haplotype diversity (*Hd*), and nucleotide diversity (π) per population were established using DnaSp version 4.00 (Rozas and Rozas 1999).

Analysis of molecular variance (AMOVA) (Excoffier *et al.* 1992) was performed to assess population genetic structure as implemented in software Arlequin 3.0 (Excoffier *et al.* 2005). The analysis was performed only for contemporary populations, and initially all samples were classified as a single group. Two *a priori* group definitions in AMOVA were computed to examine the changes in the among-group variance. These groupings were based on: 1) native population (Java, Madura, and Bali) versus introduced population (Kalimantan), and (2) "core" population (Java) versus "peripheral" populations (Madura, Bali and Kalimantan).

The analysis calculated Φ-statistics, analogues of *F*-statistics that integrate information about genetic distance between haplotypes and molecular variance components for the effects of individuals, populations and groups. A permutations approach was used to assess the significance of both Φ-statistics and the variance of the components (Excoffier *et al.* 1992).

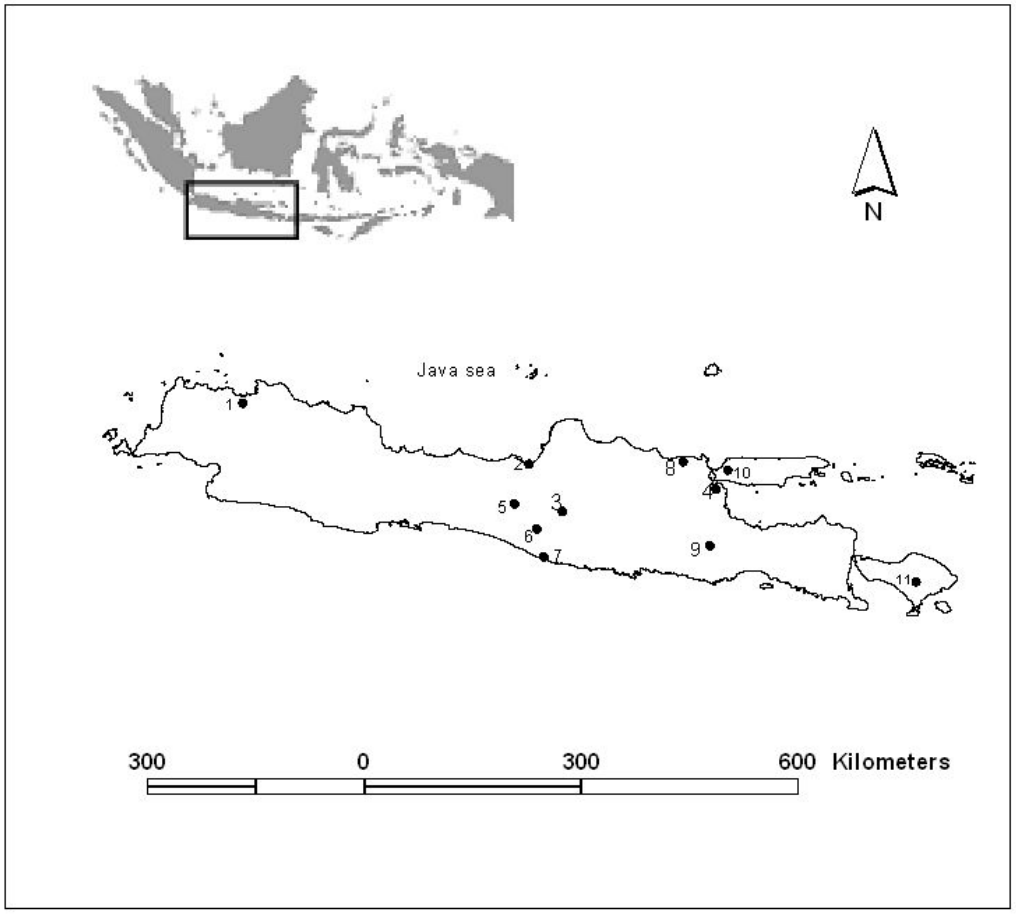


Figure 4. Sample sites for mtDNA analysis (sample sizes shown in brackets) Historical populations: 1. Jakarta (8), 2. Semarang (7), 3. Solo (7), 4. Surabaya (7). Contemporary populations: 5. Magelang (12), 6. Yogyakarta (21), 7. Gunungkidul (11), 8. Gresik (18), 9. Malang (10), 10. Madura (21), 11. Bali (11)

3.4. Results

3.4.1. Mitochondrial DNA variation

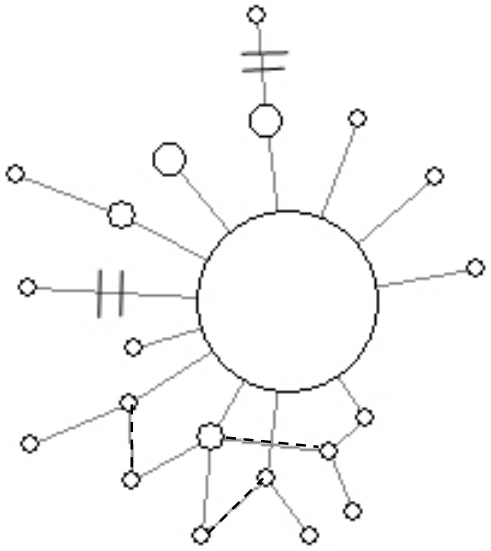
A 450 base pair region was consistently generated from all samples sequenced. Searching for similar sequences through NCBI's database (http://www.ncbi.nlm.nih.gov/blast/) revealed that Java sparrow sequences were 90-92% similar to sequences of other closely related *Lonchura* species (i.e. *L. castaneothorax*, *L. malabarica, and L. cantans* (Sorenson *et al.* 2004). Further comparisons also identified Java sparrow data as being consistent with partial sequence of control regions Part I and Part II obtained from other avian taxa (Marshall and Baker 1997).

A total of 21 haplotypes were identified from the 115 individuals sampled from contemporary populations, including the introduced population in Kalimantan. These haplotypes were described by 18 variable sites (10 transversions and 8 transitions) (Table 2). A universal haplotype (*h.1*) was observed in all 4 populations and accounted for 78% of all samples. Unique site-specific haplotypes were also found in all populations (Table 2) except Kalimantan, where no haplotypic variation was observed. The mean base composition of Java sparrow sequences was similar to that found in other avian control region studies (Baker and Marshal 1997), there being a deficiency of G (mean 16.7%) and T (25.5%), and excess of A (27.1%) and C (30.7%).

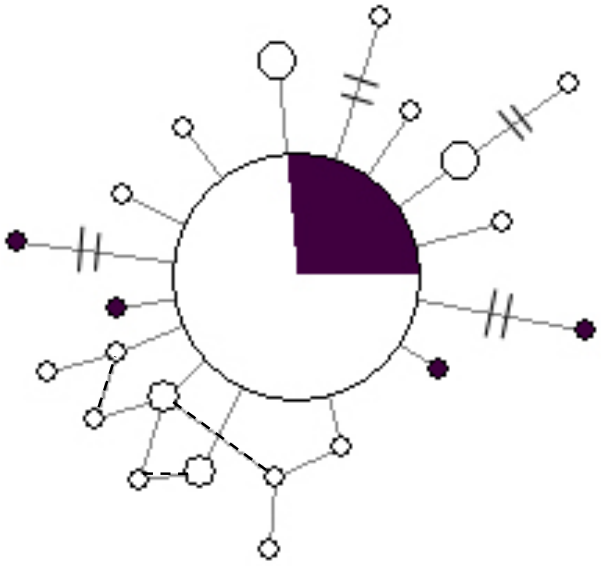
A parsimony network of base pair changes among haplotypes for contemporary populations (Figure 5) showed a 'starburst' phylogeographic pattern. Most haplotypes being separated by only a 1 bp substitution. Combining the historical and contemporary samples produced the same 'starburst' pattern. This pattern suggests that both the historical and contemporary samples belong to a population that has recently expanded from a single source population that had only a relatively small number of founder individuals (Avise 2000).

Table 2. Variability and geographical distribution of DNA control region (Part 1 and 2) sequences of contemporary Java sparrow populations
(h= haplotype; dot means as the h.1)

	Nucleotide site																		Individuals per locality				
h	77	106	130	215	217	234	305	328	332	344	361	365	366	410	411	425	433	446	Java	Madura	Bali	Kalimantan	Total
1	**T**	**T**	**C**	**A**	**A**	**T**	**A**	**T**	**T**	**A**	**C**	**A**	**G**	**G**	**C**	**C**	**T**	**A**	26	20	10	11	89
2	.	.	.	.	.	**C**	.	.	.	.	.	.	.	.	.	.	.	.	3				3
3	.	.	.	.	.	.	.	.	.	.	.	**T**	.	.	.	.	**G**	.	1				1
4	**C**	.	.	.	.	.	.	.	.	.	.	**T**	.	.	.	.	.	.	1				1
5	.	.	**A**	.	.	.	.	.	.	.	**T**	.	.	.	.	.	.	.	1				1
6	.	.	**A**	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	2				2
7	.	.	.	**C**	.	.	.	.	.	.	.	.	.	.	.	.	**G**	.	1				1
8	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	**G**	.	3				3
9	.	.	.	**C**	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	1			1
10	.	.	.	.	.	.	.	.	**C**	.	.	.	.	.	.	.	.	.	2				2
11	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	**G**	.		1				1
12	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	**T**	1				1
13	.	.	.	.	**T**		**C**	.	.	.	.	.	.	.	.	.	.	.	1				1
14	.	.	.	.	.	.	.	.	.	.	.	**T**	.	.	.	.	.	.	1				1
15	.	**C**	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1				1
16	.	.	.	**C**	.	.	.	.	.	.	.	.	**A**	.	.	.	.	.	1				1
17	.	.	.	.	.	.	.	.	.	**G**	.	.	.	.	.	.	.	.	1				1
18	.	.	.	.	.	.	.	.	.	.	.	.	.	.	**G**	**G**	**G**	.	1				1
19	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	**G**	**G**	.	1				1
20	.	.	.	.	.	.	.	**C**	.	.	.	.	.	.	.	.	.	.			1		1
21	.	.	.	.	.	.	.	.	**C**	.	.	.	**A**	**C**	.	.	.	.	1				1



(a)



(b)

Figure 5. Parsimony network for mtDNA control region Java sparrow sequences (a) contemporary populations only; (b) historical (black) and contemporary (white) population combined.

3.4.2. Historical demography

A mismatch distribution of Java sparrow mtDNA sequences (Fig. 6) was L-shaped (left truncated), again indicating that sequences from most individuals differed by a single base pair change. Raggedness indices for all populations did not deviate from the sudden expansion population model. This pattern, along with the high values of expansion coefficient (*S/d*; Table 3), again suggests that Java sparrow populations have undergone a recent rapid expansion (Harpending 1994). Further statistical tests were congruent with this finding. Tajima's *D*, and Fu's *Fs* and R_2 values were all significantly negative (Table 3). When combined these statistical tests also suggest recent population growth.

Table 3. Neutrality and other statistical tests for detecting historical demography using mtDNA control region sequences
(*n* = number of samples, *D* = Tajima's *D*; *Fs* = Fu's *Fs*; R_2 = Rozas and Rozas' test; *Ri* = Harpending's Raggedness index; *S/d* = expansion coefficient; * P < 0.05, ** P<0.001; NC: not calculated)

Locality	*n*	*D*	*Fs*	R_2	*Ri*	S/d
Madura	21	-1.1635	-0.9189	0.213	0.664	10.5
Bali	11	-1.1285	-0.4099	0.288	0.438	5.5
Kalimantan	11	NC	NC	NC	NC	NC
Java	72	-2.1585*	-20.546**	0.0299**	0.051	16.2
Pooled	115	-2.2782**	-29.225**	0.0215*	0.819	25.9

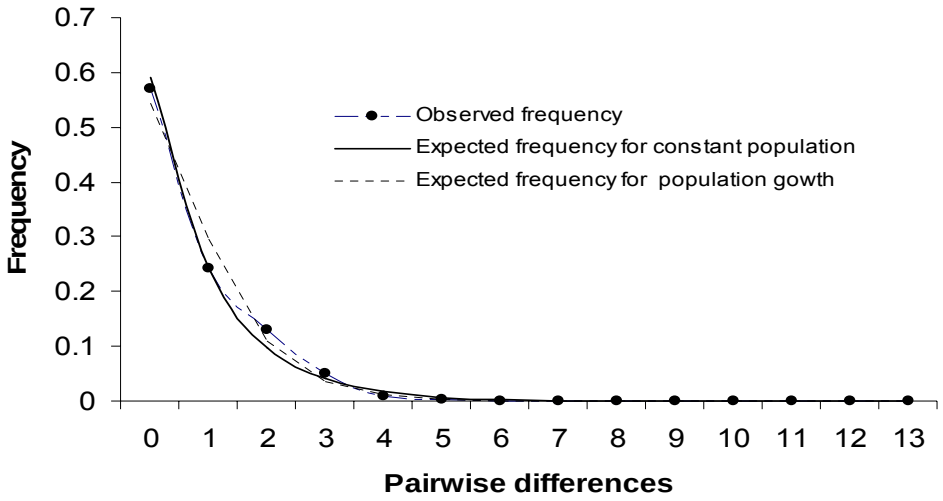


Figure 6. Mismatch distribution for Java sparrow populations in its natural range: Java Madura, and Bali. Black dots indicate observed values and white dots expected values under a sudden expansion growth model.

3.4.3. Time of expansion

The estimated divergence time between the most distant Java sparrow haplotypes suggested that the population expansion previously identified occurred between the late Pleistocene and early to mid Holocene, depending on the mutation rate used (Table 4). For the whole data set and a mutation rate of 14.8% the estimated time of Java sparrow expansion was ~15 5 ky BP (1.43 – 31.24 ky BP). For a mutation rate of 71.2%, the expansion time was estimated at ~3.2 kyBP (0.29 – 6.49 kyBP). However, more recent divergence times were obtained if analyses were conducted separately for each island, suggesting that expansion on the island of Java preceded the colonisation and expansion in Bali (Table 4).

Table 4. The estimated divergence time from mtDNA control region sequences (NC = not calculated)

Population	t (kyBP) μ = 14.8%	CI (95%)	t (kyBP) μ = 71.2%	CI (95%)
Java	12.02	0 – 35.84	2.49	0 – 7.45
Madura	3.35	0.71 – 3.35	0.69	0.15 – 0.69
Bali	2.16	0 – 2.16	0.15	0 – 0.45
Kalimantan	NC	NC	NC	NC
All	15.46	1.43 -31.24	3.21	0.29 – 6.49

3.4.4. Genetic diversity and population structure

Combining all contemporary samples gave molecular diversity estimates for the Java sparrow of 0.456 (*Hd*) and 0.00165 (π). Separate analyses for each island population showed that the Java population had the highest molecular diversity, both in haplotype number and nucleotide diversity (Table 5).

Table 5. Genetic variation of contemporary Java sparrow populations revealed from mtDNA control region sequences
(*n* = number of sample, *S* = segregating sites, *d* = mean number of pairwise differences, *H* = number of haplotypes, *Hd* = haplotypes diversity, π = nucleotide diversity, *SD* = standard deviation)

	n	S	d	H	Hd	SD	π (%)	*SD (%)*
Madura	21	1	0.09	2	0.095	0.084	0.021	0.02
Bali	11	1	0.18	2	0.182	0.144	0.04	0.03
Kalimantan	11	0	NC	1	0	0	0	0
Java:	72	17	1.05	19	0.556	0.072	0.210	0.04
Pooled	115	18	0.69	21	0.458	0.065	0.165	0.03

Table 6. Genetic variation of contemporary and historical Java sparrow populations on Java Island revealed from mtDNA control region sequences (*n* = number of sample, *S* = segregating sites, *H* = number of haplotypes, *Hd* = haplotypes diversity, π = nucleotide diversity)

Population	*n*	*S*	*H*	*Hd*	π
Contemporary	72	14	16	0.498	0.003
Historical	29	6	5	0.261	0.001
Pooled	101	20	20	0.434	0.003

Greater genetic variation was anticipated from the sequences of museum samples taken from the historical populations from before population decline, but this was not the case. Analysis of sequences from the same fragment length (310bp) of mtDNA control region revealed that the number of haplotypes observed in contemporary populations of Java sparrows in Java (*H*=16) was higher than for the historical population (*H*=5), both in total and when haplotype diversity was scaled for the relative number of samples (Table 6). Therefore these data provide no evidence that the level of diversity differs between sample types.

An analysis of molecular variance (AMOVA) for contemporary native populations revealed no significant hierarchical structuring (Table 7). Moreover, all pairwise *Fst* (Table 8) were also not significant. This suggests that based on mtDNA sequences data the Java sparrow was a single panmictic population throughout its native range.

Table 7. Analysis of Molecular Variance results for the Java sparrow for mtDNA.

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation	Fst	P
Among populations	3	0.632	- 0.005	-1.62	-0.016	0.91
Within populations	111	35.403	0.319	101.62		
Total	114	36.035	0.314			

Table 8. Pairwise Fst (below diagonal) and P (above diagonal) values

		1	2	3	4
1	Jawa		0.5869	0.93555	0.74023
2	Madura	-0.00456		0.99902	0.58594
3	Kalimantan	-0.02875	-0.03471		0.99902
4	Bali	-0.01924	0.01536	0	

3.5. Discussion

3.5.1. Molecular diversity

It has been suggested that genetic diversity is positively related to population size (Frankham *et al.* 2002) and, as a consequence, endangered species typically have low genetic diversity (Frankham 2003). The current genetic diversity of the Java sparrow is low compared to that of some endangered species, such as blue chaffinch *Fringilla teydea* (Pestano *et al.* 2000), but high relative to others, such as the black-faced spoonbill *Platalea minor* (Yeung *et al.* 2006). However, compared to closely-related passerine species of similar body size that are not endangered, nucleotide diversity of the Java sparrow was very low. For example, calculated from almost the same mtDNA fragment, nucleotide diversity in the Java sparrow (0.0018, range 0.0004 – 0.0023 control region part I & II) was ~75% of that found in the willow tit

Parus montanus (0.0023, range 0.0006 – 0.0032, ND2 and CR; Kvist *et al.*(2001), ~20% of that in the common rosefinch *Carpodacus erythrinus* (0.0044, range 0.0024-0.0026, CR; Pavlova *et al.* (2005) and ~10% of that found in the greenfinch *Carduelis chloris* (0.13, range 0.11-0.15, CR; Merila *et al,*(1997).

These findings suggest either that effective population sizes in the Java sparrow have remained smaller than those of these other sparrows and finches over relatively long periods of time, or that nucleotide diversity in the Java sparrow has been reduced by recent population declines associated with trapping. The lack of differences in the level of diversity among historic and contemporary samples suggests that any recent bottleneck has not yet significantly impacted levels of diversity. This makes the former explanation more likely, possibly because the Java sparrow is a range-restricted (endemic) species found only on relatively small islands. Similar patterns have been reported for other range-restricted species such as the blue chaffinch *Fringilla teydea* (Pestano *et al.* 2000). This is also in accordance with a general hypotheses: that species with small geographic ranges should generally have low levels of genetic diversity compared with more widespread species (Gaston 2003) such as the willow tit (Kvist *et al.* 2001) and common rosefinch (Pavlova *et al.* 2005). However, because of the small number of historic samples available, this interpretation must, for the present, remain speculative.

3.5.2. Historical demography

The starburst phylogeographic pattern of the parsimony network suggests that the Java sparrow has expanded its range relatively recently from a small or modest number of founders (Avise, 2000). The mismatch analysis revealed a highly truncated distribution in all populations, providing a generally better fit to an "expansion" rather than "equilibrium" model (Rogers and Harpending 1992). The mismatch distribution was strongly L-shaped (left truncated), similar to the greenfinch (Merila *et al.* 1997), but rather atypical when compared with studies of other finch species (e.g.Munoz-Fuentes *et al.* 2005; Pavlova *et al.* 2005).

Raggedness indices for all populations did not deviate from the sudden expansion population model. This pattern along with the high values of expansion

coefficient (*S/d*) again suggests that Java sparrow populations have undergone a recent rapid expansion (Harpending 1994). Further statistical tests were congruent with this finding. Tajima' s *D*, and Fu's *Fs* and R_2 values were all significantly negative. Combined these statistical tests also suggest recent population growth.

The results for each of the different mutation rates used in the analysis suggest two different possible historical scenarios for demographic changes in Java sparrow populations. Firstly, using a mutation rate of 14.8%, the results suggest that the bottlenecking of Java sparrow populations was likely associated with the retreat of the last glacial maxima of the Pleistocene. This scenario is consistent with the historical vegetation changes in South-east Asia, and particularly on the island of Java. During the last glacial maximum the current islands of Java, Madura, and Bali, were part of Sundaland and experienced a cooler (temperature 2.5-4° C lower) and drier climate. As a consequence, montane and savannah vegetation were more widespread, and rainforest more restricted, than at present (Heaney 1991). However, the climate changed to warm and wet at either ~ 9 – 18 ky BP (Whitten *et al.* 1997) or at ~ 5 – 11 ky BP (Kazuko Urushibara-Yoshino 1997), allowing the extensive redevelopment of rainforest. Thus, the contraction of woodland/savannah habitat during this period may have caused bottlenecking in open woodland species such as the Java sparrow. The potential cause of later expansion under this scenario is unknown and may vary depending on the estimate of when the melt occurred. This is the mutation rate used by virtually all other previous studies of this type on Northern Hemisphere taxa. This finding provides evidence that glaciation impacted species that could be expected to have had increased habitat availability during glacial maxima, in the same way that it impacted species that were range restricted during these periods. This is because of the subsequent loss of habitat during glacial retreat.

Secondly, using a mutation rate of 71.2%, the result suggests that Java sparrow populations were bottlenecked more recently during the Holocene and that the subsequent population expansion was concordant with human induced alterations to the environment on the island of Java. Fujiwara (1990 cited in Kazuko Urushibara-Yoshino 1997) reports that about 5 ky BP, forest disturbance occurred in Java, that paralleled the arrival of human immigrants from South-east Asia. These immigrants were masters of swidden and rice cultivation (Koentjaraningrat 1985),

and their arrival is thought to have resulted in the contraction of the forest and the expansion of more open habitat and cultivated areas (i.e. paddy fields).

Both estimated expansion times provide evidence that the Pleistocene land bridge may have also facilitated dispersal of the Java sparrow from Java to Madura and Bali islands, which are only recently separated islands. About 11 ky BP (Biswas 1973), sea level dropped ~120 m, reconnected Java, Madura and Bali as part of Sundaland, and seas reached their present levels only 6 ky BP (Karns *et al.* 2000).

3.5.3. Implication

This study reveals that at present populations of the Java sparrow have relatively low levels of genetic diversity, similar to those observed in other endangered species. Low genetic diversity makes the species less adaptable and therefore more susceptible to the negative genetic and demographic impacts of rapid and/or stochastic environmental change, particularly changes associated with human induced habitat deterioration. This is of particular conservation concern when total population sizes are also small and populations are highly fragmented, as is the case for the Java sparrow (see Chapter 2).

The lack of mtDNA differentiation might suggest that all Java sparrow populations are part of the same Evolutionarily Significant Unit (ESU) - as defined by Moritz (1994). Further discussion of this conservation implication is presented in Chapter 7. However, this finding must be interpreted with caution. Significant evidence of a recent population expansion means that the assumptions of standard AMOVA may be violated. If so, then much of the mtDNA signal in the data could represent historic rather than contemporary associations among populations. Hence, analysis of more rapidly evolving nuclear markers is required to make a full assessment of current levels of inter-population movement. The following chapter (Chapter 4) will discuss the use of microsatellites to reveal the current connectivity among remnant populations and the genetic diversity of the Java sparrow.

CHAPTER 4. MICROSATELLITE VARIATION AND POPULATION STRUCTURE

4.1. Introduction

As described in the previous chapter (Chapter 3), an analysis of mtDNA control region sequences inferred that there was no significant differentiation among Java sparrow populations across the species natural range. Even though mtDNA was considered as one of the most suitable markers for detecting recent isolation between populations (Zink 1997), this study revealed that the mtDNA data for the Java sparrow are likely to be indicating a residual historical signal and not contemporary gene flow. For that reason, it is necessary to evaluate the population structure of the Java sparrow using nuclear DNA markers with mutation rates that are more rapid than those of mtDNA.

Hypervariable microsatellite DNA loci are suitable for such a purpose. These simple/sort repeated tandem (1- 6 bp in size) DNA motifs are abundant, highly polymorphic in the genome, and are codominantly inherited. Moreover, microsatellites are widely regarded as neutral genetic markers. Hence, microsatellites are increasingly being used to investigate genetic variability and population structure. Several studies have provided evidence that the use of multi-loci microsatellite markers produces higher resolution signals that reveal population structuring hidden to more slowly evolving markers (e.g.Johnson *et al.* 2003).

4.2 Aims

The current distribution of the Java sparrow (Chapter 2) suggests that the large distance separating the remnant populations and small effective population sizes may have led to isolation and genetic differentiation. If so, individual populations may well have undergone bottlenecks during the fragmentation process and now be vulnerable to the effects of inbreeding.

The purpose of the study presented in this chapter is to evaluate genetic variability within and among remnant populations of the Java sparrow based on multi-loci microsatellite genotyping. By doing so, current connectivity and population structure can be revealed and applied to the development of sound management strategies for this species.

4.3. Methods

4.3.1. Sampling

I used 141 Java sparrow samples obtained from 6 wild populations and two presumed wild populations derived from the bird markets (Figure 2). These samples were collected from Magelang (MAG, n=14), Yogyakarta (YOG, n=24), Gunungkidul (GUN, n=11), Lamongan (LAM, n=20), Malang (MAL, n=17), Madura (MAD, n=21), Bali (BAL, n=13), and Kalimantan (KAL, n=21). In addition 51 samples from a museum collection were also included so that the genetic status of the Java sparrow prior to any potential decline over the last 50 years could also be examined. The museum samples included specimens collected from 4 sites across Java, i.e. Jakarta (n=10), Semarang (n=10), Solo (n=10), and Surabaya (n=10) (Figure 2; Appendix 3), and an introduced population from the Cocos-Keeling Islands (n=13). These samples were obtained from historical museum specimens collected during the period 1932-1941 (Appendix 3), prior to any perceived decline. The detail sampling method and DNA extraction procedure have been described in Chapter 3.

4.3.2. Microsatellite typing

Five microsatellite loci were used to genotype each Java sparrow sample. The primers used were Indigo28, Indigo28, BF2, BF3, and BF18, which were originally developed for *Vidua chalybeata* (Sefc *et al.* 2001) and *Lonchura striata* var *domestica* (Yodogawa *et al.* 2003) respectively (Table 9).

Table 9. The primers used to amplify the Java sparrow

Primer	Annealing temperature (ºC)	Sequences	Repeat array
Indigo27	55	F: FAM-GAGGTATTTCTGCCCCACTAT R: GACCCAATGCTGTATGGC	$(TG)_4(TA)_4(CA)_{14}$
Indigo28	55	F: FAM-CCCAGGAAGTATCCCAGAA R: CCTCCAATGCTTTAGTGACC	$(ATG)_{16}$
Bf18	60, 58, 55	F: TET-GGTGGTGCGTGGTGAGAGTA R: TCACCCCGGATTCTAGCACG	$(GT)_2GA(GT)_6GC(GT)_9$
BF2	60, 58, 55	F: FAM-GCCTAAAGAGTATCCCATGA R: AAATCTCCCACAACCCCCT	$(CAAA)_8$
BF3	60, 58, 55	F: HEX-GGCTTAGCAGACAGCTTTGG R: GGAACAAGCAGCCAGCAC	$(CA)_{10}AA(CA)_{20}$

Each polymerase chain reaction (PCR) was carried out in a 10 µl reaction volume containing 10x PCR Buffer (200mM Tris-HCL (pH 8.4), 500mM KCl); 2.5mM $MgCl_2$; 5pmol of fluorescently labelled forward primer; 5pmol of unlabelled reverse primer; 0.1 mM of each dNTP and 1 unit of *Taq* polymerase (Life Technologies). PCR conditions for the Indigo27 and Indigo28 loci were 5 minutes denaturing 94ºC, 35 cycles (30s at 94ºC, 20s at 55 ºC, 30s at 72 ºC) and final extension 72 ºC for 5 minutes. The BF 2, 3 and 18 loci were amplified using a 'step-down' annealing procedure: 5 minutes denaturing 94ºC, 4 cycles (30s at 94ºC, 20s at 60 ºC, 30s at 72 ºC), 4 cycles (30s at 94ºC, 20s at 58 ºC, 30s at 72 ºC), 22 cycles (30s at 94ºC, 20s at 55 ºC, 30s at 72 ºC) and final extension 72 ºC for 5 minutes.

The amplification products were purified using ethanol precipitation and screened using MegaBACE™ DNA Analysis Systems at the Genetic Analysis Facility, James Cook University, Townsville.

4.3.3. Statistical analysis

4.3.3.1. Genetic variation and population structure

Genetic diversity was measured using several parameters such as mean number of alleles across multi loci (k), allelic richness (A), and observed (H_O) and expected heterozygosities (H_E). These parameters were calculated using the software Genalex 6 (Peakall and Smouse 2006). Allelic richness was computed following Petit *et al*. (1998) implemented in FSTAT (Goudet 1995). A non-parametric Friedman test was used to compare the level of genetic diversity among populations.

The Hardy-Weinberg equilibrium test was performed using a single locus and a global multi-locus test for heterozygote deficiency, or excess, as applied in the program GENEPOP web version of 3.4 (Raymond and Rousset 1995). This program was also used to examine linkage disequilibrium. Estimates of exact *P*-values for these tests were calculated by the Fisher's exact test. Default dememorization number (1000), 100 batches and 1000 iterations per batch were applied for all calculation based on Markhov-chain models. Sequential Bonferroni correction (Rice 1989) was applied to correct for multiple simultaneous comparisons across loci. In order to estimate the levels of non-random association of alleles within populations, inbreeding coefficients (F_{IS}) were calculated for locus specific and overall values using FSTAT (Goudet 1995).

Analysis of molecular variance (Excoffier *et al.* 1992) was computed to assess the level of genetic variation within and among populations. The level of population genetic differentiation was examined using *F*-statistics (Nei 1977). Among-population variance in allelic frequencie (F_{ST}) was calculated using the software Arlequin ver. 3.0. (Excoffier *et al.* 2005). The significance of F_{ST} value was tested using a non-parametric permutation approach described in Excoffier et al. (1992), performed in Arlequin with 1000 of permutations for the significance ($\alpha = 0.05$).

4.3.3.2. Effect of geographic distance on genetic distance

To assess the effect of interpopulation distance on levels of gene flow between populations, a series Mantel tests was implemented for specific

combinations of populations: (1) the contemporary populations (including the introduced population of Kalimantan). (2) the natural contemporary populations (excluding the Kalimantan population), and (3) the natural historical and contemporary populations. I tested the relationship between genetic distance and direct geographic distance between each pair of study populations for these data sets. The genetic distances used were the pair-wise *F*st, and the geographic distance was measured as direct distance calculated from Lat/Long coordinates applied in GenAlEX 6.

4.3.3.3. Bottleneck detection

A recently bottlenecked population will display a reduction in both heterozygosity and allele numbers. However, according to Maruyama and Fuerst (1985), the reduction in allele numbers will happen faster than the loss of heterozygosity. Based on this trend, excess heterozygosity in a population at mutation-drift equilibrium has been used to develop a test to detect a recent population decline (Cornuet and Luikart 1996). This approach was used to analyse microsatellite data from the Java sparrow, using the software BOTTLENECK (Cornuet and Luikart 1996). The assumption used in the program is that the distribution of genetic diversity expected from the observed numbers of alleles is in mutation-drift equilibrium. Various possible mutation models were applied to calculate the expected average heterozygosity (H_{EQ}). These models included Infinite Allelic Model (IAM), Single Stepwise Mutation Model (SMM), and two-phased model of mutation (TPM). The program was run in default parameter setting and 1000 replications. To determine the significance of the heterozygote excess, the Wilcoxon sign-rank was used, following the authors' recommendation for the number of loci and sample size of this study.

4.3.3.4. Detecting migrants and estimate migration rate

A genetic assignment test was used to detect first generation migrants. The test uses genotype likelihood to assign or exclude each individual to a particular population and so identify the population that it is most likely to have come from. This also allows a direct real-time assessment of dispersal (Paetkau *et al.* 2004). This assignment test was performed using software GENECLASS 2 (Piry *et al.* 2004),

which is based on Bayesian methods (Rannala and Mountain 1997). *P* values were calculated with Monte Carlo re-sampling following simulation using 1000 iterations. Wilson and Rannala (2003) also used this non-equilibrium approach to develop a method to estimate the rates of recent migration among populations. This Bayesian multi-locus genotyping method allows genotype frequencies to deviate from Hardy-Weinberg equilibrium proportions within populations (Wilson and Rannala 2003; Pearse and Crandall 2004). Moreover, compared to the other methods to estimate long-term gene flow, this method requires fewer assumptions, and is therefore more appropriate for this study due to the evidence of Pleistocene expansion of the Java sparrow (Chapter 3).

Migration rates among the Java sparrow populations were also estimated using the computer program BAYESAS (Wilson and Rannala 2003). The posterior probability of migration rate was estimated by running a Markov Chain Monte Carlo simulation for a total of $3x10^6$ iterations and discarding the first 10^6 iterations as burn-in. To infer the posterior probability distribution, samples were collected every 2000 iterations.

4.4. Results

4.4.1. Genetic diversity

Table 10 summarizes the mean number of alleles, allelic richness, and the observed and expected heterozygosities. All 5 loci were polymorphic in all populations. A total of 59 alleles were observed across the 5 loci and 141 individuals from contemporary populations. There was an average of 11.8 alleles per locus, ranging from 6 alleles (BF2) to 16 alleles (Indigo28). The mean number of alleles per locus per population ranged from 5.8 (SE=1.4) to 8.4 (SE=1.5). The non-parametric Friedman test indicated that both allelic richness and observed heterozygosity were not significantly different among remnant contemporary populations (X^2= 9.905, *df*=7, *P*=0.194; X^2= 3.139, *df*=7, *P*=0.872, respectively).

Only 4 loci consistently amplified in the historical samples, and BF2 worked poorly for unknown reasons. In total 45 alleles were found at 4 loci, with an average of 1.25 alleles per locus, ranging from 6 (BF3) to 18 (Indigo18). For the

historical populations the non-parametric, Friedman test also indicated that both allelic richness and observed heterozygosity did not differ significantly among populations (X^2= 3.235, *df*=4, P=0.519; X^2= 3.68, *df*=4, *P*=0.451, respectively).

Since historical samples were only collected from Java and only 4 loci were amplified, comparison analysis to contemporary populations was undertaken using only contemporary samples collected from the same island and amplified with the same 4 loci. This analysis included 86 samples from contemporary populations and 40 samples from historic ones. The observed heterozygosity of the historical populations was not significantly different from that of the contemporary populations (non-parametric Wilcosox sign test, *P*=0.06; *Ho*=0.78 and 0.64 for historical and contemporary populations respectively). There was also no indication of significant differences in either the allelic richness (*P*=0.44; *A*=10.8 and 10.4 respectively), or the mean number of alleles (*P*=0.89; *k* = 13 and 10.8 respectively).

Table 10. Microsatellite diversity indices of the Java sparrow
(n: sample size; k = average number of allele per locus; A= allelic richness; H_o, H_e: observed and expected heterozygosities averaged over loci, ** P< 0.01)

Population	*n*	k	*A*	H_o	H_e
Contemporary populations:					
Yogyakarta	24	8.4	6.7	0.61**	0.73
Gunungkidul	11	6.2	6.2	0.509**	0.712
Magelang	14	5.8	5.5	0.486**	0.683
Lamongan	20	7.2	5.8	0.540**	0.724
Malang	17	6.8	5.7	0.635	0.663
Madura	21	7.2	6.1	0.562**	0.724
Bali	13	6.4	6.0	0.585**	0.669
Kalimantan	21	7.8	6.4	0.657**	0.727
Historical populations:					
Jakarta	10	4.4	4.9	0.620	0.520
Semarang	10	4.4	5.7	0.720	0.565
Solo	10	5.4	4.9	0.551	0.544
Surabaya	10	5.2	5.3	0.600	0.659
Cocos-Keeling Island	11	4.4	4.3	0.777	0.592

4.4.2. Hardy-Wienberg and linkage equilibrium tests

The global multilocus test to detect Hardy-Weinberg equilibrium (HWE) revealed that all of the contemporary Java sparrow populations but with the exeption of Malang had highly significant heterozygote deficiencies ($P<0.01$) compared to the expected values (Table 10). This contrasted with findings for all historical populations where genotype frequencies did not depart from HWE. The single locus Hardy-Weinberg tests (per locus per population) indicated that 67% of the tests performed revealed significant heterozygote deficiency at $P <0.05$. These deficiencies were distributed across all loci and populations with no consistent patterns among populations or loci obvious. No locus showed a significant heterozygote excess. The pattern did not change after Bonferroni correction except for the Malang population, where all loci did not deviate from Hardy-Weinberg.

The linkage disequilibrium test revealed that genotypes at all loci were independent of each other. Only three pairs of comparisons were significant out of 80 tests, but after sequential Bonferroni correction all test were not significant (Table 11).

Table 11. P values of linkage equilibrium test between loci across populations

	Populations							
Locus	YOG	GUN	MAG	LAM	MAL	MAD	BAL	KAL
Ind27 X ind28	0.102	1	0.016	0.057	0.007	1	0.218	1
ind27 X bf18	0.243	0.349	0.253	0.792	0.079	0.366	0.529	0.128
ind27 X bf3	0.941	0.246	0.660	0.659	0.176	0.948	0.546	1
ind27 X bf2	0.559	0.826	0.098	0.060	0.886	0.555	0.921	0.411
ind28 X bf18	0.214	0.133	0.249	0.425	0.067	1	0.048	0.807
ind28 X bf3	1	1	1	0.753	1	0.632	0.321	0.687
ind28 X bf2	0.981	1	0.886	0.149	0.524	0.066	0.555	0.7
bf18 X bf3	0.903	1	0.629	0.751	1	0.926	0.241	0.982
bf18 X bf2	0.875	0.744	0.129	0.599	0.989	0.677	0.357	0.448
bf3 X bf2	0.750	0.313	0.247	0.989	0.331	0.398	0.372	0.564

4.4.3. Inbreeding coefficient

Inbreeding coefficient values (F_{IS}) for each locus by population combination ranged from 0 to 1 (Table 12). Calculations for overall loci by population showed different levels of inbreeding across native populations and introduced populations of Java sparrows. The Malang population experienced the lowest level of inbreeding (0.07), and the Gunungkidul population experienced the highest (0.328). This finding corresponded with the fact that most populations deviated from Hardy-Weinberg Equilibrium (see section 4.4.2). Using limited data, only four populations which have both data (inbreeding coefficient and population size), it is likely that the level of inbreeding is negatively correlated with population size (Figure 7)

Table 12. F_{IS} values for each locus by population

	YOG	GUN	MAG	GRE	MAL	MAD	BALI	KAL
Indigo27	0.426	0.144	0.441	0.439	-0.341	-0.041	0.294	0.072
Indigo28	0.211	0.375	0.12	0.191	0.338	0.203	0.108	0.172
BF18	0.05	0.521	0.393	0.178	-0.152	0.265	-0.129	0.159
BF3	0.262	-0.198	0.133	-0.031	0.202	0.088	-0.215	-0.091
BF2	-0.15	1	0.722	0.728	0.328	0.913	1	0.348
All	0.192	0.328	0.323	0.275	0.071	0.247	0.165	0.12

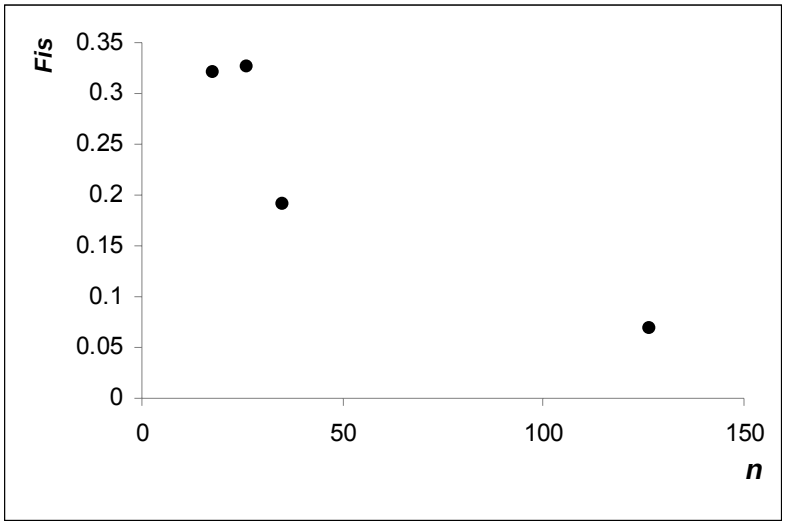


Figure 7. Correlation between inbreeding coefficient and population size of four Java sparrow populations

4.4.4. Population differentiation

The AMOVA revealed that most of the observed genetic variation is explained by variation within (96.15%) rather than among populations (3.85%). Even so, the variation among populations led to significant genetic structuring with limited differentiation (F_{ST} = 0.038; $p < 0.001$). Moreover, highly significant pairwise F_{ST} values were found between all Java sparrow populations on the island of Java, except between Gunungkidul and Magelang (Table 13). Pair-wise F_{ST} values were not significant between the introduced population from Kalimantan and the three native populations from Lamongan, Madura, and Bali. Bonferroni correction also revealed no significant differences between these paired populations.

Incorporating the historical sample into the analysis revealed that the pair-wise F_{ST} values between historical populations were not significantly different, except for those paired with the introduced populations from the Cocos Islands. However, the historical populations were significantly different from all contemporary populations (Table 13).

4.4.5. Isolation by distance

Using the first data set, of the contemporary populations including the introduced population from Kalimantan, the Mantel test revealed no relationship between geographic distance and genetic distance (P=0.339). However, excluding the introduced population from Kalimantan from the analysis (second data set) showed a weak but significant relationship between geographic and genetic distance (P=0.038). The test based on the natural historical and contemporary populations (third data set) was marginally non-significant (P=0.057). Overall these findings indicated that weak isolation by distance effects may occur among Java sparrow populations across their natural distribution.

4.4.6. Population bottleneck

The analyses used to detect bottlenecking produced inconsistent results. Under the assumption of the infinite alleles model (IAM), 4 populations (Yogyakarta, Gunungkidul, Magelang, and Madura) showed significant heterozygote excess (He>Heq) (Table 14). Under the two-phased model (TPM), the Magelang and

Madura populations were the only samples to display significant deviance. Under the step-wise mutation model (SMM) none of the populations showed significant deviance from mutation-drift equilibrium with heterozygote excess. The obvious evidence of an historic expansion wave in the mtDNA data set may also violate the assumptions of this analysis, weakening its ability to detect recent bottlenecks.

Table 13. Pair-wise values of F_{ST} among the Java sparrow populations (below diagonal), and values (above diagonal) between all pairs of populations (contemporary: 1-8; historical: 9-13; bold : not significantly different, $p \geq 0.05$)

Populations	1	2	3	4	5	6	7	8	9	10	11	12	13
1.Yogyakarta		0.024	0.001	0.011	0.008	0.001	0.001	0.001	0.000	0.001	0.000	0.000	0.000
2.Gunungkidul	0.025		0.121	0.034	0.002	0.034	0.008	0.005	0.000	0.000	0.000	0.000	0.000
3.Magelang	0.067	**0.014**		0.003	0.001	0.001	0.001	0.001	0.000	0.004	0.000	0.004	0.000
4.Lamongan	0.023	0.029	0.038		0.019	0.027	0.001	0.136	0.002	0.002	0.000	0.006	0.000
5.Malang	0.025	0.053	0.072	0.022		0.001	0.001	0.001	0.002	0.001	0.001	0.008	0.000
6.Madura	0.048	0.028	0.039	0.018	0.058		0.005	0.108	0.000	0.000	0.000	0.002	0.000
7.Bali	0.042	0.038	0.058	0.009	0.049	0.011		0.181	0.000	0.000	0.000	0.000	0.000
8.Kalimantan	0.049	0.044	0.093	**0.052**	0.074	**0.042**	**0.009**		0.000	0.000	0.000	0.000	0.000
9.Jakarta	0.079	0.124	0.096	0.059	0.064	0.086	0.154	0.092		0.661	0.076	0.679	0.000
10.Semarang	0.041	0.074	0.050	0.047	0.053	0.070	0.117	0.073	**-0.009**		0.439	0.410	0.061
11.Solo	0.092	0.121	0.093	0.092	0.091	0.137	0.179	0.131	**0.032**	**0.001**		0.049	0.147
12.Surabaya	0.053	0.087	0.058	0.041	0.044	0.055	0.126	0.073	**-0.009**	**0.001**	**0.037**		0.000
13.Cocos Is.	0.086	0.139.	0.141	0.101	0.116	0.142	0.189	0.131	0.097	**0.037**	**0.017**	0.095	

Table 14. Departures from mutation-drift equilibrium in the Java sparrow populations (The P=values are for Wilcoxon sign-rank (one-tailed) tests for the heterozygote excess (He>Heq), and are shown for expected distribution of heterozygosity estimated under IAM, TPM and SMM

Populations/ Sampling sites	IAM	TPM	SMM
Yogyakarta	0.046*	0.593	0.953
Gunungkidul	0.031*	0.406	0.812
Magelang	0.015*	0.031*	0.406
Lamongan	0.078	0.687	0.953
Malang	0.406	1.000	1.000
Madura	0.015*	0.031*	0.968
Bali	0.109	0.593	0.953
Kalimantan	0.312	0.312	1.000

4.4.7. Migration assessment

The assignment test found that a total of 4 out of 141 samples were possible first generation migrants. Three of these migrants originated from Kalimantan and were observed in Yogyakarta, Lamongan (Java) and Bali. The other one was from Bali, and was detected in Malang (Java).

The averages of the posterior distribution of **m** (the migration rate into each population) are summarised in Table 15 Two populations (Magelang and Malang) were likely relatively isolated. There were four distinctly large average migration rates: the average migration rate from Malang to Lamongan 0.23, from Malang to Madura 0.22, from Malang to Bali 0.21, and from Malang to Kalimantan 0.27. Malang and Lamongan are relatively close to one another, but the remaining populations were not. Some are not only separated by long distances but are also located on different islands. Migration patterns between populations were also asymmetrical, with the migration rate into Malang being lower. This implies that the Malang population was a genetic source population for these other locations.

Table 15. Migration rates (m) among Java sparrow populations. Standard deviations for all distributions were < 0.05.

	The origin of the migrants							
	YOG	GUN	MAG	LAM	MAL	MAD	BAL	KAL
YOG	*0.787*	0.007	0.010	0.007	0.173	0.008	0.007	0.007
GUN	0.052	*0.700*	0.085	0.016	0.093	0.022	0.015	0.014
MAG	0.006	0.006	*0.929*	0.005	0.033	0.005	0.005	0.006
LAM	0.014	0.007	0.032	*0.685*	*0.233*	0.009	0.007	0.009
MAL	0.005	0.003	0.006	0.003	*0.969*	0.004	0.003	0.003
MAD	0.014	0.007	0.024	0.007	*0.219*	*0.716*	0.007	0.007
BAL	0.017	0.010	0.033	0.010	*0.208*	0.015	*0.692*	0.012
KAL	0.007	0.005	0.012	0.006	*0.267*	0.007	0.006	*0.685*

4.5. Discussion

4.5.1. Genetic diversity

The level of genetic variability for the Java sparrow, expressed as the mean number of alleles per locus, allelic richness, and observed or expected heterozygosity was in the middle range for values observed in other endangered birds. Compared to other declining bird species with wide distributions, such as the Yellowhammer (*Emberiza citronella*) in the United Kingdom (Lee *et al.* 2001), and the Cerulean warbler (*Dendroica cerulea*) in North America (Veit *et al.* 2005), the level of genetic variability for the Java sparrow was lower. However, when compared to endangered species which inhabitant small islands, or to those that consist of a single or a few small populations (*i.e.*: Laysan finch (Tarr *et al.* 1998), Loggerhead shrike (Mundy *et al.* 1997), Mariana crow (Tarr and Fleischer 1999), it was higher. Thus, it would appear that the level of genetic diversity for the Java sparrow is still relatively high given its current distribution, in spite of the recent population declines over the last three decades (IUCN 2006).

Theoretically, a declining population will experience a loss of genetic diversity (Frankham 1995). However, although the level of genetic diversity in the

Java sparrow was low, the population decline during the last three decades has not caused a loss of genetic variation as compared to levels observed in the historical samples, either in allelic richness or heterozygosity. This is likely due to the relatively rapid and recent nature of the declines. However, it could be expected that genetic diversity in this species will continue to decline if effective population sizes remain low for extended periods of time.

The presence of heterozygote deficiency in all remnant populations (except Malang) but not in equivalent historic population samples, suggests that non-random mating associated with mechanisms of sexual selection and mate choice may already be influencing levels of genetic variation. The inbreeding coefficients (see Section 4.4.3) support this argument. Another possible explanation is that the Java sparrow has experienced intense genetic drift at each location due to stochastic demographic processes. Drift is expected to be more intense in small populations. Census results (Chapter 2) support this argument. Allele frequencies in the largest population (Malang) did not deviate from Hardy-Weinberg, while those in the remaining smaller populations did.

Other biological phenomena that may produce such deficiencies are the presence of non-amplifying (null) alleles at the microsatellite loci used (Allendorf and Luikart 2007) and/or undetected population structure (Wahlund effect) within sampling locations. Both possibilities are unlikely. The presence of null alleles is unlikely due to the locus unspecific nature of the heterozygote deficiencies observed across both loci and sampling locations (Allendorf and Luikart 2007), while unresolved population structuring within such small populations can be considered the equivalent of non-random mating.

4.5.2. Genetic structure

The Java sparrow used to be a common 'pest' species throughout its natural range in Java and Bali. The current population study (Chapter 2) found that Java sparrows are now scarce and that their distribution is highly fragmented. AMOVA using multi-locus microsatellite data revealed significant genetic structuring among the remnant populations (F_{ST} = 0.038; $p < 0.001$). This level of genetic structuring

was high when compared to microsatellite data for similar widely distributed passerine species. For example, the Yellowhammer (*Emberiza citronella*) across the UK (F_{ST} = 0.010) (Lee *et al.* 2001), the Yellow warbler (*Dendroica petechia*) across Canada and Alaska (F_{ST} = 0.014) (Gibbs *et al.* 2000) and the Pied flycatcher (*Ficedula hypoleuca*) between Norway and Spain (F_{ST} = 0.0275), and between Spain and the Czech Republic (F_{ST} = 0.0246) (Haavie *et al.* 2000).

It has been suggested that weak or no population genetic differentiation is a common feature for many bird species, most likely due to high rates of dispersal (Crochet 2000). On the other hand, the level of differentiation found for Java sparrow populations in this study suggested that gene flow between sites was limited. Additionally, highly significant pairwise F_{ST} values were also found between most of the remnant Java sparrow populations on the island of Java, while contrary to this, F_{ST} values between the introduced population in Kalimantan and those in the three other major regions Lamongan, Madura, and Bali were not significantly different. This finding suggests that genetic drift has a stronger influence on allele frequencies than gene flow among the contemporary Java sparrow populations. Furthermore, that interconnectedness or gene flow was limited is further supported by the presence of isolation by distance effects.

Incorporating historical samples into the analysis revealed that historically it is likely that the Java sparrow was panmixic. Pairwise F_{ST} for most of the historical samples was not significant, even though these samples were of equivalent size and collected from a greater geographical range (see Fig 2). This finding suggests that recent population decline has led to differentiation among the remnant populations and genetic fragmentation.

4.5.3. Migration

On average the migration rate among sites, as measured using assignment test, was low, ranging from 0.007 to 0.267 (Table 4.6). This finding is consistent with the AMOVA and isolation by distance analyses. Among site migration was asymmetrical, and suggested that the Malang population was a likely source population for other sites, particularly for the eastern part of the Java sparrow distribution. This finding highlights the possibility of long-distance dispersion of the Java sparrow between relatively isolated populations. However, this is unlikely

without dispersal also occurring into intermediate populations. Moreover, morphological analysis on the ratio of wing-length to the cube root of body weight, an index that has previously been considered a good indicator of dispersal ability, suggests that the Java sparrow is not a long-distance disperser. This ratio (x=2.3, SD=0.1, N=20) was far smaller in Java sparrows than in Gouldian, Masked, and/or Long-tailed finches (x=8.5, SD=0.6, N=10; x=7.1, SD=0.4, N=10; x=6.5, SD=0.5, N=10; respectively), species that are all known to be medium to long-distance dispersers. This makes human mediated movement through the bird markets the most likely explanation (see Chapter 5 for further detail).

4.5.4. Implications

In concordance with the low level of mtDNA variation (Chapter 3), the microsatellite data also provides evidence that at present Java sparrow populations have a relatively low level of genetic diversity, even though the recent population decline seems to have had a limited impact on microsatellite diversity. This finding is of particular conservation concern when the major threat of trapping still continues at high levels (see Chapter 5).

Moreover, significant genetic structuring of the remnant populations indicates limited connectivity among populations only due to recent fragmentation and genetic drift. Hence, for genetic management of the Java sparrow, the remnant populations should be considered as a single management unit (MU), as defined by Moritz (1994). Further discussion of the conservation implications of this finding are presented in Chapter 7.

CHAPTER 5. THREATS ASSESSMENT

5.1. Introduction

In general, habitat loss, fragmentation, and degradation are believed to be the primary threats to wild populations of birds on a global scale. However, there are also many other threats, which may, or may not be mutually exclusive to habitat loss, or indirectly caused, or enhanced by habitat degradation. These threats include overexploitation of species by hunting and the pet trade, invasive species, climate change, and novel zoonotic diseases (Primack 2002; Sodhi and Brook 2006). For example, 34 bird species in Asia are threatened with extinction due to capture for the pet trade (BirdLifeInternational 2001).

In Indonesia bird-keeping is a popular pastime, with deep cultural roots (Jepson and Ladle 2005). It is widely assumed that the hobby negatively affects wild populations of common as well as threatened birds (Nash 1994; Jepson and Ladle 2005). Moreover, the Indonesian government's capacity and willingness to implement wildlife regulations is limited (Reeve 2002). Not surprisingly, about 20% of 104 Indonesian endangered bird species are affected by this overexploitation (Shanaz *et al.* 2000), including the Bali Mynah (*Leucopsar rothschildi*), Purple-napped lorry (*Lorius domicella*) and Java sparrow (*Padda oryzivora*).

The spread of virulent pathogens can also have devastating demographic effects and significant impacts on the overall fitness of surviving individuals. Examples include: the great rinderpest pandemic that swept through African wildlife after importation of domestic cattle (Scott 1981), the appearance and subsequent spread of duck plague throughout North American waterfowl population (Friend and Peasson 1973,), and the introduction of avian malaria and pox virus to Hawaiian Islands (Dobson and May 1986). A study on blood parasite prevalence in forest birds in South-east Asia found that over 50% of the examined bird species were parasitized by more than one species (Paperna *et al.* 2005). However, little has been known about the level and prevalence in non-forest birds in this area.

5.2. Aims

In this chapter I will discuss findings on the level of the major threat to the Java sparrow, i.e. exploitation for the pet trade. The level of trapping was assessed by surveying trappers, and the level of trade was tracked by investigating movements of birds in the markets.

Secondly, I will explore another potential threat which comes from disease, i.e. avian malaria. Molecular assessment was applied to assess the prevalence level of avian malaria in the wild population of Java sparrow.

Thirdly, I will discuss the other threats based on the available secondary data and references.

5.3. Methods

5.3.1. Market survey

In order to assess the continuing level of threat from trapping and trading, bird market surveys and further interviews with bird trappers were carried out. These surveys were conducted during January to July and October to December 2004 and gathered information on the number of Java sparrows for sale at each market and their provenance. The survey sites included 6 bird markets in East Java and 5 bird markets in Central Java (Table 16; Fig. 5.1). In these markets, only 1 to 4 shops provided Java sparrows for sale. Direct counts were carried out to estimate the number of the birds for sale in all shops in the markets. To gain information about the provenance of the birds, I interviewed the shopkeepers. Birds were classified, as either captive bred or wild caught based on the origin of the sites. For all wild caught birds, interviews with trappers obtained data on trapping sites, methods used, and the level of trapping frequency and success per unit effort. A total of seven bird trappers were interviewed, two from Surabaya and five from Yogyakarta (including Gunungkidul).

Table 16. The sites of the bird markets survey (N = the number of shops providing the Java sparrow for sale)

Sites	N
The province of Central Java:	
1 . Semarang	1
2. Yogyakarta	
a. Ngasem	3
b. Kalasan	2
3. Wonosari	1
4. Solo	1
The province of East Java:	
5. Bandarjo	1
6. Malang	3
7. Surabaya	
a. Bratang	2
b. Tumpang	3
c. Turi	3

5.3.2. Avian malaria assay

5.3.2.1. Samples

In total, 38 DNA samples, randomly chosen from the samples used for the population study (see Chapter 4 & 5), were used to assess the prevalence of avian malaria in Java sparrow. As a comparison, two common finches species, i.e. Chesnut munia (*Lonchura ferruginosa*) and White-headed munia (*Lonchura maja*), were also assessed wirh 15 samples being used for each.

5.3.2.2. Molecular analysis

I used a nested-PCR assay developed by Hellgren et al. (2004) which enabled one to detect three common genera of blood parasites in parallel, including Haemaproteus, Plasmodium, and Leucocytozoon. The protocol involved a two step PCR. Firstly, to amplify the cytochrome-*b* of these three genera, PCR was performed in volumes of 25 µl, which included ~50 ng of total DNA, 1.25 mM of each

deoxynucleoside triphosphate, 1.5 mM $MgCl_2$, 0.6mM of each primer, and 0.5 units Tag DNA polymerase. The primers used were HaemNFI (5'– CATATATTAAGAGAAITATG GAG – 3') and HaemNR3 (5'-ATAGAAAGATAAGAAATACCATTC-3').

PCR was conducted for 20 cycles under the following conditions: 94° C for 30 sec, 50° C for 30 sec, and 72° C for 45 sec. The samples were incubated before cyclic reaction at 94° C for 3 min and after cyclic reaction at 72° C for 10 min. The product of this PCR was used as a template for the second PCR step, respectively 1 µl for *Haemoproteus* spp*., Plamodium* spp. and for *Leucocytozoon* spp. The primers used to amplify the former parasites were HaemF (5'-ATGGTGCTTTCGATATATGCATG – 3') and HaemR2 (5'-GCATTATCTGGATGTGATAATGGT –3') (Bensch *et al.* 2000). Meanwhile, the primers for the latter were HaemFL (5'- ATGGTGTTTTAGATACTTACATT – 3') and HaemR2L (5'- CATTATCTGGAT GAGATAATGGIGC – 3') (Hellgren *et al.* 2004). This PCR was run separately in 25µl with the same proportion of reagents as in the first PCR reactions. The thermal condition of the PCR was as for the first PCR except for 35 cycles instead of 20 cycles. To ensure consistency of the result, 15 of the samples were run three times.

Final PCR products were visualized with electrophoresis, by loading 5 µl of the products and 2 µl of loading dye (Bromophenol Blue) onto a 2% agrose gel. Ethidium bromide (EtBr) was included into the gels to visualise the DNA. Gels were run in x1 TBE buffer at 45 MA for approximately 25 minutes.

The positive samples were then selected for sequencing either using primer HaemF (for *Haemoproteus* spp.- *Plamodium* spp.) or HaemFL (for *Leucocytozoon* spp.). Double strand PCR products were purified by ethanol precipitation or spin column purification (Ultra Clean Tm, MO BIO Inc), prior to cycle sequencing using DYEnamic ET Dye Terminator Kit (MegaBACE). Sequencing products were purified and screened using MegaBACE™ DNA Analysis Systems at the Genetic Analysis Facility, James Cook University, Townsville. Identification of parasites was determined by searching for similar sequences through the NCBI's database (http://www.ncbi.nlm.nih.gov/blast/).

5.4. Results

5.4.1. Trapping

Table 17 shows the level of trapping in each of the study sites. Catch rates per unit effort by bird trappers are equal to, if not better than, the success rate obtained during the mark-recapture study over a similar period (see Chapter 2). This scale of trapping is alarming. The numbers obtained by bird trappers are clearly greater than the remnant populations observed in the areas where trapping occurred.

Table 17. Number of birds caught in 2004 as specified by bird trappers

Site	Period	Catch effort	No. of Bird caught		
			Adult	Juvenile	Total
Prambanan	April - May	6	13	2	15
Kepurun	July – Sept.	9	14	9	33
Jothak	April	1	NA	NA	22
Lamongan	May	5	32	44	76
Dadapan	July	2	6	5	11

Note: NA = data unavailable

The results from the interviews with trappers revealed that Java sparrows were mostly caught in feeding areas, either in paddy fields (Prambanan and Kepurun) or in dry agricultural areas (Lamongan, Dadapan). However, in Jothak the birds were trapped on or around their nest sites. The trap methods used varied among sites. In Prambanan and Kepurun double clap-nets were used; meanwhile single clap-nets were used in Lamongan and Dadapan. Both methods used live decoy birds. A mistnet was used in Jothak. In other sites in Gunungkidul, along with a live decoy bird, *Arthorcarpus* gum was used to trap the birds. To increase the adhesiveness of the gum, powder of the root of 'Ragen' or 'Gerip putih' (*Parameria barbata*) was mixed into the gum.

This study also revealed that the trapping was done during Java Sparrow breeding time. Trapping was particularly intense when parent birds with fledglings were feeding in the paddy field just after the harvesting time, from March to July.

5.4.2. Market survey

In total, the estimated numbers of the Java sparrow available for sale in the markets during the period of this study were 1905 birds (Table 18). These estimates were for two different periods of time. The first period of the study (January to July) resulted in 737 birds and coincided with the breeding period of the birds in Java. Most of the birds caught during this period were extracted from local populations from various sites in Java. In contrast, the second estimate obtained during October to December (1168 birds), contained mostly imported birds from other islands, a large portion of the latter were derived from birds in the Surabaya markets (i.e. Bratang, Turi, and Kupang). The trappers indicated that the large number of birds available during the final months of the year was due to a high demand from the Chinese community, who have a custom of releasing birds at New Year.

Table 18. The numbers of the Java sparrow for sale in the markets and their provenance

Market location	Provenance of the birds		
	Captive bred	Wild populations	Unknown
East Java:			
Bratang	4	599	15
Turi	-	457	58
Kupang	163	-	80
Malang	116	98	24
Bandarjo	-	10	-
Central Java:			
Semarang	85	-	-
Magelang	31	-	-
Solo	21	-	-
Ngasem	34	41	-
Kalasan	-	39	-
Wonosari	-	30	-
Total	454	1274	177

Overall the provenance of the birds available for sale in the market was mostly supplied from wild birds (67%), and the remaining were from those which were bred in small scale captive breeding industries (24%) and unknown sources (9%)(Table

18). Analysis per region resulted in slightly different proportions for East Java (72%, 17% and 11 % respectively), but not for those of Central Java.

The wild birds offered in the bird markets came from various sites (Figure 8), including some areas which are not the natural range of the Java sparrow (i.e. Kalimantan and the Sumbawa Islands). Interestingly, these areas along with Madura, provided the primary sources of the birds for sale in the Javanese markets (Fig. 8a). This was particularly true for the bird markets in East Java. Analysis per region revealed that in the East Java bird markets, 83 % of the birds were derived from other islands and only 17% from local sources (Fig. 8b). In contrast, in the bird markets in central Java, the local wild birds were the only stock available for sale (Fig.8c). Furthermore, this study also found that the Surabaya markets played a major role as the market gateway for Java sparrows imported from the outer islands and shipped to the Javanese markets elsewhere. From Surabaya the birds were distributed to other cities in Java, such as Malang, Solo, and Semarang (Fig. 9).

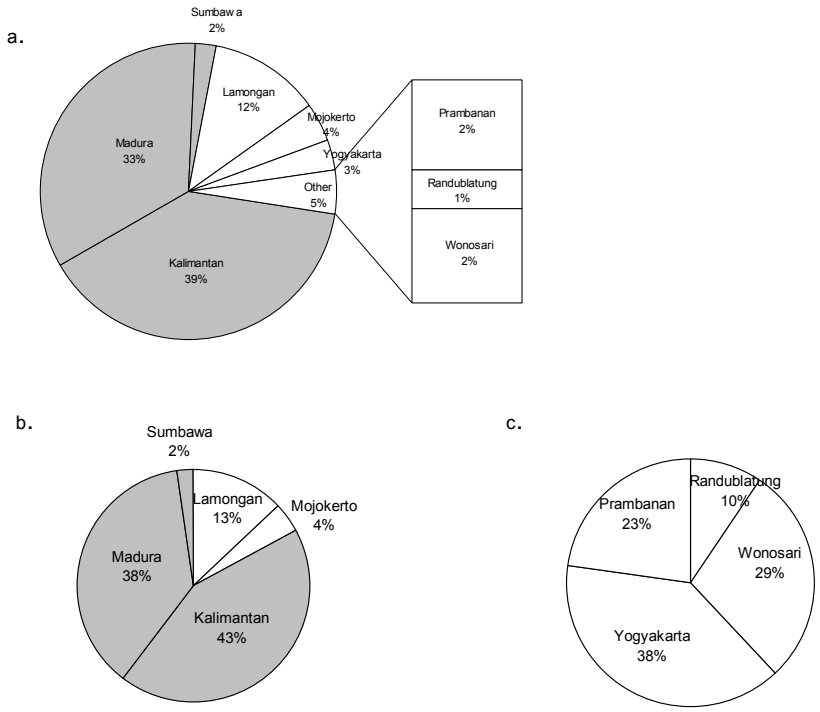


Figure 8. The provenance of the wild birds offered in the bird markets: a. overall data, b. East Java, and c. central Java. Grey area is imported birds, and white area is local birds.

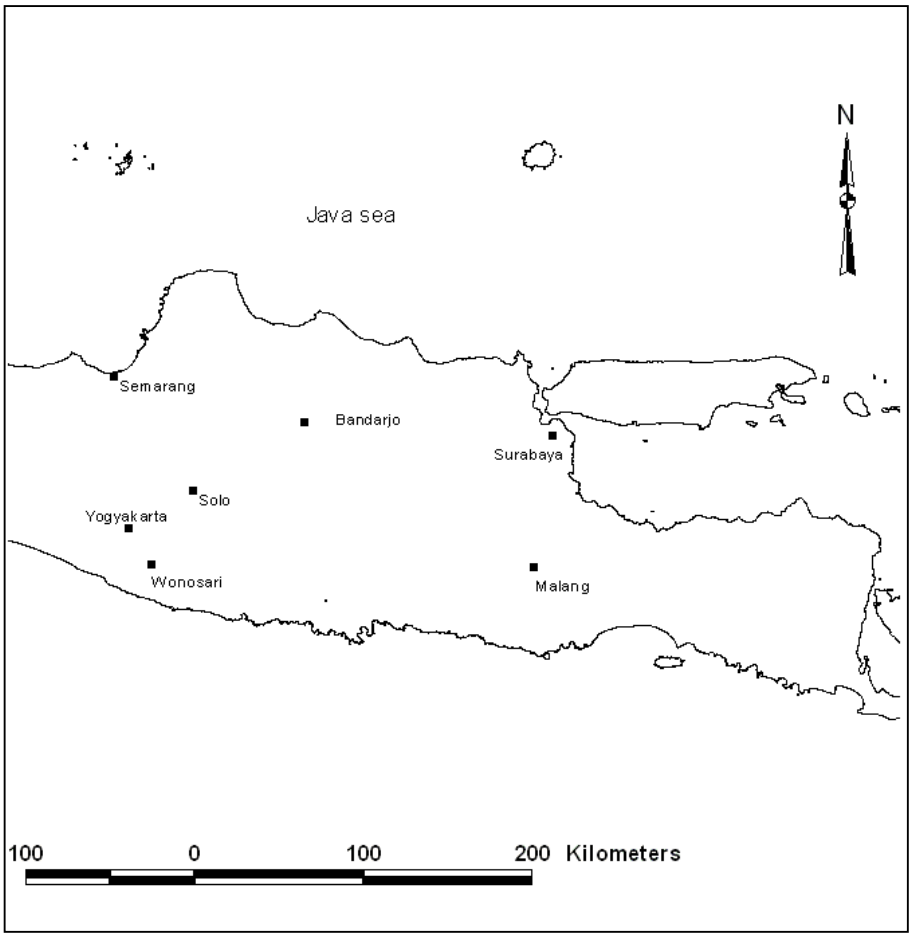


Figure 9. Map of the market survey and the trafficking of the Java sparrow

5.4.2. Detection of avian malaria

The PCR assay positively detected *Haemaproteus-Plasmodium* parasites in 11 out of 38 Java sparrow blood samples (28.95%), but no positives for *Lyucocytozoon* in the same samples. Meanwhile, neither of the blood parasites was

detected in the Chesnut munia (*Lonchura ferruginosa*) or in the White-headed munia (*Lonchura maja*). The repeatability test consistently produced the same results.

About 450 base pair regions were consistently generated from all positive samples sequenced. Searching for similar sequences through NCBI's database (http://www.ncbi.nlm.nih.gov/blast/) revealed that both *Haemoproteus* and *Plasmodium* were present in the infected Java sparrows' blood. The prevalence of infection of the former parasite (23.68 %) was higher than the latter (5.26%).

The sequences of *Haemoproteus* resulting from this study have a 97-99 % similarity with the published sequences of the same genus in the NCBI's database; and slightly smaller (92-96%) similarity for those of *Plasmodium*. These findings suggest new haplotypes of avian malaria specific to the Java sparrow, consisting of 3 haplotypes of *Haemoproteus* (h.1-3) and 2 haplotypes of *Plasmodium* (h.4-5) (Table 19).

Table 19. Haplotypes of avian malaria found in the blood of the Java sparrow (*h:* haplotype; *n*: number of samples)

h.	Sequence	n
1	CTCTTAACCTTTTCCACTTTATTTTAACATTTTTTCTTCCTTATGATACTCCCACTC	6
2	CTTATAACTTCTTACACTTTATTTTAACATTTTCTATCCCTTATGATAATCTCATTT	2
3	CTTTTAACTTCTTCCACTTTATTTTAACATTTTCTATCCCTTATGATAATCTCACTT	1
4	TTTTTTTTTATAAACTCATTATAATTCTGACTCTCAATTTAAGCATATTCATTATAT	1
5	TCTTCTATTATTTTTATTACCATTCTCTGACATTCAATCTAAGCAAATTTTTTTTAT	1

5.5. Discussion

5.5.1. Trapping and trading

Trapping Java sparrow for the caged-bird trade is suspected to be the main factor causing the observed decline of this species (BirdLifeInternational 2001). Individually caged Java sparrows continue to be in high local demand for socio-cultural reasons, particularly in Yogyakarta. Local people place a high value on

being able to consume captive Java sparrows obtained from Prambanan as one of the requirements of the '*mitoni*' (7th month of pregnancy) ritual. Wealthy members of the Chinese community release birds, including Java sparrow, on special occasions (e.g. weddings, New Year).

This study confirmed that this threat continues to have an impact Java sparrow populations. Overall the number of birds for sale in the markets found in this study was greater than the total observed remnant population in east and central Java (Chapter 2). This was possible because of the importation of birds from other islands, including birds from the natural populations on Madura and introduced populations established in Kalimantan and Sumbawa. Given the continuing high demand for the Java sparrow at certain times of year, it is unlikely that local stocks can fulfill the demand. Meanwhile, during mid-year, the market demand is likely covered by capture of birds from populations in Java during the peak of the breeding season (March till July) and from those derived from captive breeding.

The results suggest Surabaya market was a likely transit point for Java sparrows sold in the local trade. Birds imported from Kalimantan or other outer islands appear to firstly be pooled in Surabaya before being distributed to other cities in Java (i.e. Malang, Solo, and Semarang). In contrast, Muchtar and Nurwatha (2001) reported that Semarang was the gateway for the trafficking of Java sparrows. Despite this discrepancy, both findings imply an abundance of Java sparrows on those islands, particularly Kalimantan. However, to date no population studies have been conducted in these areas.

During this study, three sites provided data on both remnant populations and the level of trapping/capture in 2004. These were Prambanan, Kepurun and Jothak. The level of trapping was very high with an average of 53.6%, 47.9%, and 70.9% respectively of the projected pre-trapping population for that year being removed. The average of the level of trapping for adult birds was 38%. If this level of trapping continues, the population of the Java sparrow will undoubtedly collapse in the short term. This conclusion is discussed further in Chapter 6.

The high rate of harvest in Yogyakarta is mainly because of the high demand, which makes harvesting of Java sparrows lucrative for local trappers. It might be said that they harvest Java sparrows in their own backyard. In contrast, for trappers in east

Java, harvesting Java sparrows is no longer profitable. They have to travel further and need more time to achieve catches, meaning increased operational costs.

These findings imply that minimizing further trapping should be the first priority of reducing the decline of this species. The protection of roosting and nesting sites is the first step in an effort to save the Java sparrow. The Malang and Prambanan birds, though not in a nature reserve, profit by indirect protection from the sites' authorities. Despite this protection, trapping still occurred at these sites in associated feeding areas (i.e. paddy fields), where Java sparrows are generally considered a pest. Although the area is private land, the owners or people living in the surroundings areas, do not prevent bird trappers depleting local populations. This was also true for the Jothak population, which experienced a more intense exploitation at the nesting site. For this reason it is very important to involve local people in conservation initiatives for the Java sparrow. This may include encouraging the local people to initiate small scale breeding projects. It is likely that existing commercial breeding already significantly mitigates the extraction of birds from wild populations. Further promotion of commercially-bred alternative birds has been suggested as an effective and popular solution to the high demand for birds in Indonesia (Jepson and Ladle 2005).

In addition to strengthening the above initiatives, the Java sparrow should also be listed as a protected species. It is surprising that the Java sparrow has not apperared on the list of Protected Species in Indonesia. However, local conservation initiatives have already been taken by local government (e.g. Surabaya) and local community groups (e.g. awig-awig in Bali). Other local governments and/or communities should follow these initiatives. Such initiatives are very important, as most of the remnant populations are not in nature reserves or other protected areas in Indonesia.

5.5.2. High prevalence of avian malaria infection

This study found that the prevalence of avian malaria in the Java sparrow was very high compared to levels in the two other common Indonesian finch species assayed in this study, and also high compared to those of forest birds in Java (Paperna *et al.* 2005). Using the blood smear method, the latter study found that of

152 birds from 27 species assayed for the prevalence of infection 4.3 - 17 % and 0 - 0.4 % tested positive for *Haemoproteus* and *Plasmodium* respectively, depending on the habitat types. Birds living in lowland forests of Java seem to be more susceptible to infection than those in upland forests (Paperna *et al.* 2005).

This finding suggests that the Java sparrow is more prone to parasite infection, i.e. avian malaria, compared to the more common finch species. This factor could be another potential threat, increasing the risk of extinction for this species. However, the impact of this infection on the demography of Java sparrow is so far unknown. A controlled experiment may need to be set up to quantify this potential impact.

5.5.3. Other threats

For Java sparrow, other factors have been suggested as threats, i.e. competition with Eurasian tree sparrow (*Passer montanus*) and intensive use of pesticides (Balen 1997; BirdLifeInternational 2001). The following sections will discuss these threats based on the available data and references.

a. Competition with Eurasian tree sparrow

Many references suggest that there are ecological similarity between the Java sparrow and the Eurasian tree sparrow (Balen 1997; BirdLifeInternational 2001). For this reason the occurrence of the two species in the same habitat may result in interspecific competition. However, closer examination of the niche requirements suggests that competition between the two species is more likely to be over nesing sites than food resources. The differences on bill shape and size suggest the differences suggest that there are some differences in preferred food types. In addition, the principal food resources used by both species, such as paddy seeds are very abundant. Likewise, previous studies have found that competition for nest sites between Java sparrow and Eurasian Tree sparrow did occur, but was not consistently present at all locations. For, example, evidence from Sukawati (Bali), Sukabumi (West Java) and Malang (East Java) clearly suggested the two species compete (Muchtar and Nurwatha

2001), but no nest site competition was observed in the Prambanan temple complex (Fanny *et al.* 2006) or in Malang (*pers. obs*.) where the two species co-exist. These premilinary findings identify nest site competition is a peripheral threat and further studies are needed to gain a more comprehensive understanding of the importance of this factor in the decline of the Java sparrow.

b. Intensive use of pesticide

Pesticide has been used intensively in the paddy system of Java as a means of enhancing agriculture production. But as has been shown elsewhere intensive pesticide use also has a cost to the environment, including impacts on bird species. The declines of some raptor populations in North America and Europe have been correlated with organochlorine insecticide (OC) contamination (Mineau 1999). Furthermore Grue *et al.* (1983) reported that between 1965-83 acutely toxic cholinesterase (ChE)-inhibiting organophosphate (OP) and carbonate (CB) pesticide have killed 52 species from 15 bird families.

In Indonesia DDT was used widely and is now prohibited. Despite this, farmers still regularly obtain and use it illegally. The only studies on the insectivorous bird edible-nest Swiftlet (*Collocalia esculenta*) suggest that this bird has been contaminated by pp-DDD (0.09 ppm) (Laudensius *et al.* 2003), and organophosphate (Kuncoro *et al.* 2002). However, the impact of this contamination on the reproduction and population demography of the swiftlet is still unknown. Given that the cumulative effect of pesticide on insectivorous birds is most likely higher than on seed-eating birds, it is believed that the Java sparrow experiences less contamination by pesticide than the swiftlet.

5.5.4. Implications

This study revealed that the major threat of trapping for trade is still continuing at a high level. Even though captive bred birds and imported wild birds from introduced populations have a major role in market supply, it is unlikely that this importation reduces the level of trapping of wild populations, particularly for the remnant populations in central Java. Population modelling to assess the impact of

trapping is discussed in Chapter 6. If the current trapping continues it is expected that the Java sparrow will become extinct within a relatively short period of time. Alternative conservation actions to overcome this problem are also discussed in Chapter 7.

Current data suggest other threats, particularly from nest competition and pesticides, may be occuring but that these are more peripheral. However, data are scare and to gain a more comprehensive understanding of the relative influence of these factors on ecology of the Java sparrow, further studies are necessary.

CHAPTER 6. POPULATION VIABILITY ANALYSIS

6.1. Introduction

Population viability analysis (PVA) is a process proposed by Gilpin and Soule (1986) to estimate the effects of various events on the likelihood that a population will become extinct. The analysis ranges from qualitative and verbal processes to simulation models that examine the dynamics of a population or metapopulation (Akcakaya 2000; Reed *et al.* 2002). This tool can be used to investigate the relative importance of different reproductive or demographic factors on population viability and to gain an improved understanding of the causes of extinction based on species-specific data (Akcakaya 2000; Frankham 2002). This means that PVA can be used to integrate and examine all of the potential factors affecting the extinction probability of particular species. So far PVA is the most common form of risk assessment used for endangered species (Gilpin and Soule 1986; Caughley and Gunn 1996).

The other potential use of PVA is in guiding management decisions for small and threatened populations (Morris and Doak 2002), with respect to: planning fieldwork (Akcakaya 2000), designing reserves (Shaffer 1981; Armbruster and Lande 1993), identifying key life stages or demographic processes as management targets (Crouse *et al.* 1987), planning a release program (Armstrong and Ewen 2002), deciding the population number needed to protect a species (Lindenmayer and Possingham 1996), assessing human impact (Morris and Doak 2002), and/or as an instrument of moderation in discussing conservation problems (Seal *et al.* 1998; Burgman and Possingham 2000).

Basically, two types of PVA modelling processes and associated computer software have been developed; these are species-specific and generalized PVA packages. Species-specific models are generally more expensive, time consuming, to develop and cannot be reused (Brook *et al.* 2000). By comparison, generalized models can be applied to any species and are open to examination, evaluation, and repeated improvement . Various PVA computer programs are commercially or freely

available, among them VORTEX, GAPPS, INMAP and RAMAS . These have made it relatively easy to carry out PVA as part of a conservation assessment. Several hundred PVAs have been conducted for endangered species (Seal *et al.* 1998; Menges 2000), with VORTEX being the most widely used package by the Conservation Breeding Specialist Group of the IUCN (Lacy 1993; Seal *et al.* 1998). For the last two decades the Conservation Breeding Group of the IUCN have assessed more than 70 endangered species (http://www.cbsg.org/reports). In Indonesia, PVAs have been performed in order to aid with the conservation of many endangered species, including the Orangutan (*Pongo pygmaeus*), Komodo monitor (*Varanus komodoensis*), Javan Hawk-eagle (*Spizaetus bartelsi*) and Bali Starling (*Leucopsar rothschildhi*).

6.2. Aims

The aims of this chapter are:

1. To implement a PVA to assess the potential fate of remnant populations of Java sparrows based on the demographic and other life history data available.
2. To perform a sensitivity analysis to identify parameters which have especially strong effects on population growth
3. To asses the potential impact of trapping on the population dynamics of the remnant populations.

6.3. Methods

A PVA for the Java sparrow was performed using the software VORTEX ver. 9.61 (Lacy 1993). The program was designed to model species with low fecundity, a long lifespan and low local population size (N<500), and is capable of incorporating genetic effects into the modelled data set. Because of its general applicability, this program has been widely used and is a well-tested software package for population viability analysis (Bustamente 1996; Hoyle *et al.* 1998; Brook *et al.* 2000).

Sensitivity analysis is particularly useful in achieving the aims of this chapter, as it measures the effect of changes in the input parameters on the modelled populations viability (i.e. the risk of extinction and/or population growth rates). Although the actual results from population modelling should be treated with caution, the model outcomes can help in identifying parameters that need to be estimated as accurately as possible or, if data is limited, that need further research in order to obtain accurate estimates (Burgman *et al.* 1993).

6.3.1. Data

Detailed data on the breeding biology of Java sparrows in the wild are very limited. Prior to the work outlined in this thesis, published studies on the Java sparrow have focused mainly on overall species distribution, potential threats and the associated implications for conservation (e.g. Balen 1997; Muchtar and Nurwatha 2001). Therefore, the data used in this analysis had to be derived from multiple independent sources: this study (Chapter 2), a published study on wild and captive Java sparrows, data from captive birds obtained from personal communications with aviculturists, and data available on the most closely related species, i.e. Scaly-breasted munia (*Lonchura punctulata*) (Sharma *et al.* 2004). These multiple data sources were then used to derive best estimates for further PVA analysis. The following sections explain in detail how these estimates were derived.

1. Breeding system

Observation of three pairs of banded birds in Prambanan during the 2004-2005 breeding seasons revealed that the Java sparrow was most likely socially monogamous. Each breeding pair formed and remained together for at least one breeding cycle.

2. Breeding age

Data on the breeding ages of wild Java sparrows is limited to the small amount of data obtained from the Prambanan field site during this study, in which banded birds started breeding at twelve months of age. In captivity individual birds mostly breed between the ages of 1 to 4 years; however breeding ages as young as 8 months and as

late as 8 years have also been observed. In this PVA analysis the minimum and maximum breeding ages were set at 1 and 4 years respectively.

3. Sex ratio

In this study the sex of adult Java sparrows was determined based on morphological characteristics, while molecular sexing techniques were used for chicks (Chapter 2). The sex ratio of juveniles tended to be female-biased with 1.5 females to 1 male, while the sex ratio for adults tended to be male-biased with 1.2 males to 1 female. However the sex ratios for both chicks and adults did not differ statistically from a 1:1 sex ratio (Chapter 2). The proportion of breeding females was assumed to be 50%.

4. Fecundity

Observations of breeding behaviour for wild Java sparrows revealed that it was likely that birds would lay up to three clutches during each breeding season. The average number of eggs per clutch was 5.5 (± 0.91, n =4) (Chapter 2), and the average fledgling number was 4 (± 2, n =3; Kurniandaru, 2006 pers.com.). This finding is similar to the average clutch size over three years of observation for the Spotted munia in an urban habitat (5.6 ± 0.93) (Sharma *et al.* 2004), as well as that observed for the Gouldian finch (5.2 ± 1.3) (Tidemann *et al.* 1999). Similarly, the average number of fledglings was also within the ranges observed for the Spotted munia (2.28 ± 1.93) and the Gouldian finch (4.8 ± 1.5)(Tidemann *et al.* 1999).

5. Annual mortality

The survival rate of the Java sparrow was calculated by the equation: Sn = 100 [%KTBA (2 month after banding) + %KTBA (3 months after banding) + %KTBA (n after banding)]/ [%KTBA (1 month after banding) + %KTBA (2 months after banding) + %KTBA (n-1 after banding)], where n is the number of months of the study and %KTBA is the percentage of individuals known to be alive (Caughley 1977; Woinarski and Tidemann 1992). For the Java sparrow the %KTBA were derived from re-sighted banded birds in successive months following banding. The mortality rate for the Java sparrow derived from this assessment was 60.3%.

Alternatively the mortality rate can also be estimated from the ratio of juveniles to adults in the population. Mortality rate (q) is equal to the number of birds (j) that are born out of a sample of (k) birds (q = j/k). Based on this approach, the mortality rate for the Java sparrow is estimated at 23% (2004 data) and 24% (2005 data).

These mortality rate estimates were low compared to those for the Gouldian finch determined using the same approach: 99% and 81%, respectively (Woinarski and Tidemann 1992). Another finch species, Cassin's finch (*Curpoducus cussiniione*), has a more comparable adult mortality rate (36 - 40%) (Mewaldt and King 1985). However, there are no data available on chick mortality in the wild. In captivity, chick mortality is less than 10% (Garrie Landry, *pers.com*. 2006). A study on wild populations of the most closely related species, Scaly-breasted munia (*Lonchura punctulata)*, found that the mortality of fledglings was 51.4% and 68.4 %, for the birds which nest in urban habitat and those which nest in forest habitat respectively (Sharma *et al.* 2004). These mortality rates are high compared to fledgling mortality in the Gouldian finch, 37.3% (Tidemann *et al.* 1999) Values encompassing all of the variations outlined above were used in the PVA analysis.

6. Initial population size

The minimum total population estimate for Java sparrow derived from roost counts at six different sites was 214 (Chapter 2). This estimate was used as the initial population size in this PVA analysis. This assumes panmixia among these populations. Population analysis using multi-locus microsatellite markers revealed limited gene flow among remnant Java sparrow populations (Chapter 4). For this reason, viability analysis was also undertaken using population estimates for individual populations, or sites, at four locations, Yogyakarta (45), Gunungkidul (26), Magelang (17) and Malang (126) (Chapter 2), using scenarios both with and without connections among the populations (see also next section).

7. Dispersal

Field data about the dispersion of Java sparrow has not been available. In this study the current dispersion among populations was derived from the molecular

analysis using multi-locus microsatellite genotyping (Chapter 4). Table 20 summarizes the probability of dispersion per generation among the four populations.

Table 20. The probability of dispersal (%) among the populations from source populations (rows) to recipient populations (columns)

	Recipient population			
	Yogyakarta	Gunungkidul	Magelang	Malang
Yogyakarta		5.2	0.6	0.5
Gunungkidul	0.7		0.6	0.3
Magelang	1.0	8.5		0.6
Malang	17.3	9.3	3.3	

8. Inbreeding depression

Data on inbreeding depression in the Java sparrow are not available. However, results from this study (Chapter 4) imply that some remnant populations of Java sparrows may be inbred. The standard values of lethal equivalent (3.14) and those of percent of the total genetic load due to the recessive lethal (50) are those recommended by the authors of VORTEX. This value is the average level found for juvenile survival in 40 captive mammalian populations (Ralls *et al.* 1988). However, since inbreeding depression affects all components of the life cycle and is typically greater in wild environments than in captive ones, O'Grady et.al (2006) argue that this value is an underestimate and have proposed a lethal equivalent of 12.3. The latter value is derived from a meta-analysis of inbreeding depressions in 10 bird and mammal species in wild habitats and includes the effects of inbreeding on fecundity, first year survival, and survival to sexual maturity. Hence, 12.3 is considered a more realistic value for inbreeding depression in wild populations (O'Grady *et al.* 2006) and was therefore used in this analysis. A default of five lethal equivalents due to recessive lethal alleles, and seven due to deleterious alleles of small effect which consist of three lethal equivalents to fecundity and four lethal equivalents to survival was used as suggested by O'Grady *et al.* (2006).

9. Harvesting

Among the Java sparrow study sites, three sites provided estimates of both remnant population size and level of trapping in 2004; Prambanan, Kepurun and Jothak (Chapter 2 & 5). The level of trapping was very high with an average of 38% of the projected pre-trapping total adult bird population for that year being removed (Chapter 5). This value was used to assess the impact of trapping on the viability of the remnants Java sparrow populations. I also performed sensitivity analysis on the level and interval of harvesting to estimate the extent of trapping that would allow populations to maintain their current status.

10. Carrying capacity

The Java sparrow is adapted to both rural and urban habitat, which likely supplies unlimited resources. Carrying capacity was initially subjectively set to 1000, changing the carrying capacity to larger values had no significant effect on the results obtained.

6.3.2. Modelling scenario

The limited availability of reliable data is a general problem for endangered species. To identify whether less reliable parameters are important in the dynamics of a population, a sensitivity analysis should be performed by examining a range of values for these parameters (Lacy 1993). In this study, sensitivity analysis was applied to less reliable parameters, including fecundity and annual mortality rates. The parameters and range of values for the sensitivity analysis are summarized in Table 6.2. Based on these values, four scenarios were set up using a set of combinations of these parameters (Table 6.3). The baseline model was set up with the assumptions of high fecundity and low mortality rate, and without inbreeding depression. In order to assess the impact of inbreeding depression, a lethal equivalent of 12.3 was also established in all scenarios.

The simulations were run for 100 years, for 1000 iterations, and used the following assumptions:

- Extinction definition : only one sex remain
- Type of mating system : monogamous
- Age of first offspring (both for male and female) : 1 year
- Maximum breeding age : 4 years

- Maximum number of progeny : 24
- Sex ratio (in %male) : 50
- Adult female breeding (%) : 50
- Maless in breeding pool : 100
- Distribution of number of offspring per female per year : normal distribution
- Age distribution : stable age distribution
- Environmental variance : 10%

Assessment on the impact of the trapping was carried out by incorporating the current level of trapping of adult birds (38%) in the baseline scenario (A), and using the following assumptions:

- First year of harvest : 1
- Last year of harvest : 100
- Interval between harvest : 1 year

In order to assess the management options (i.e. level of trapping allowed for sustainable use), a sensitivity analysis on the level of harvest was carried out based on the baseline scenario with the values of level of trapping ranging from 10% to 30% of population size, and using the same assumptions described above.

Table 21. Input data for sensitivity analysis of this study

		Parameter	Values
A. Fecundity			
1	Low	Number of offspring per female per year	2.8 ± 1.9
2	High		4.8 ± 1.5
B. Mortality rate			
1	Low	Annual mortality of fledgling (%)	37
		Annual mortality of adult (%)	23
2	Moderate	Annual mortality of fledgling (%)	45
		Annual mortality of adult (%)	41.5
3	High	Annual mortality of fledgling (%)	53
		Annual mortality of adult (%)	60.3

Table 22. The scenarios for the sensitivity analysis for the Java sparrow

Scenario	Fecundity		Mortality		
	Low	High	Low	Moderate	High
A. Baseline		✔	✔		
B. Moderate mortality		✔		✔	
C. High mortality		✔			✔
D. Low fecundity	✔		✔		

6.4. Results

The annual population growth rates were relatively high for all populations (~0.38), under the baseline scenario. The probability of the remnant populations of the Java sparrow going extinct over a short period of time (100 years) was less than 5%, for all remnant populations, either as local isolated populations or as a meta-population. In contrast, under the worst case scenario (scenario D) all populations had negative population growths and went extinct, with a mean time to extinction of 4.2 and 35.2 years for the local populations and metapopulation scenarios, respectively (Table 23).

6.4.1. Sensitivity analysis

Altering the mortality rate on the model significantly affected population growth (Fig. 10 & Table 23). Moderate levels of mortality did not change the probability of extinction, but a high rate of mortality in the baseline scenario resulted in negative population growth and a 98% probability of extinction. A similar pattern of affects was observed on population growth (Fig. 10 & Table 23) and probability of extinction (increased by 23%) with changes in the level of fecundity.

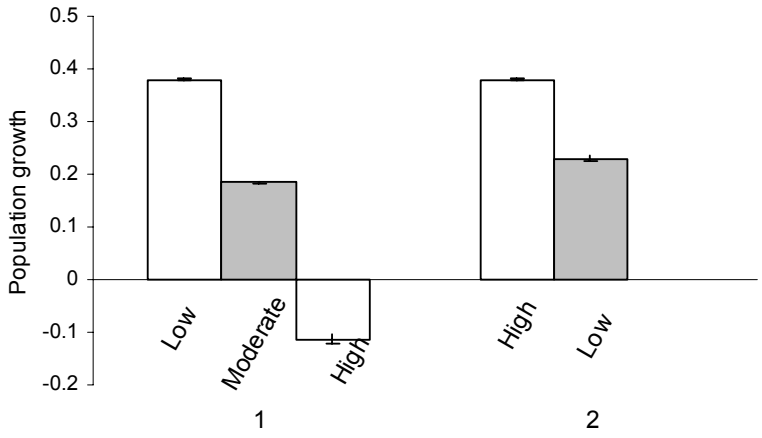


Figure 10. The effect of the level of mortality rates (1) and fecundity (2) on the population growth (±SE) of the Java sparrow population

Table 23. Sensitivity analysis – effects of changes in input parameters on model outcomes

		NoDispersion			With dispersion		
Population	Scenario	r	PE	MeanTE	r	PE	MeanTE
Gunungkidul	A	0.375	0	0	0.436	0	0
	B	0.176	0.016	12.3	0.208	0	0
	C	-0.130	0.999	14.3	-0.075	1	23.5
	D	-0.035	0.846	29.1	0.108	0.001	33
Yogyakarta	A	0.377	0	0	0.371	0	0
	B	0.176	0.002	13.5	0.14	0	0
	C	-0.119	1	20.5	-0.129	1	19
	D	-0.025	0.717	40.4	0.048	0.001	26.3
Magelang	A	0.375	0.002	5.5	0.215	0	0
	B	0.173	0.086	11.1	0.036	0	12.4
	C	-0.135	1	10.9	-0.154	1	9.5
	D	-0.043	0.923	22.5	0.016	0.001	16.3
Malang	A	0.376	0	0	0.002	0	0
	B	0.178	0	0	-0.015	0	16.4
	C	-0.109	0.999	31.4	-0.512	1	7.5
	D	-0.014	0.381	58.8	-0.021	0.016	13
Metapop	A	0.380	0	0	0.329	0	0
	B	0.184	0	0	0.149	0	0
	C	-0.113	0.998	35.2	-0.153	1	26
	D	-0.010	0.23	67.8	0.072	0.001	35

Dispersion increased growth in the Gunungkidul population, but decreased growth in the remaining populations and in the metapopulation as a whole (Fig. 11 & Table 23). In this case, dispersion had a negative impact on the source populations. This impact was highly significant for Malang, which is the source population for all others but receives few migrants (see Table 23).

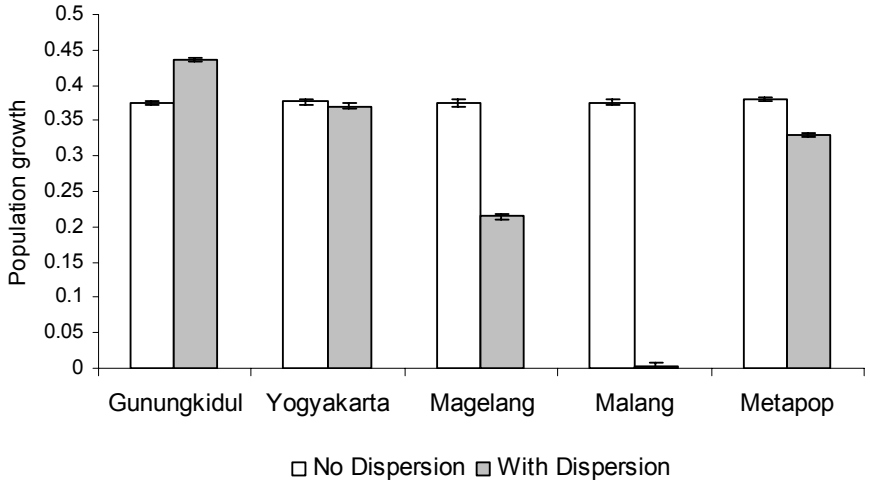


Figure 11. The effect of dispersion on the population growth (±SE) of local populations and metapopulation

6.4.2. Effect of inbreeding depression

Under the baseline scenario, incorporating inbreeding depression (LE=12.3) into the model significantly decreased population growth in all populations (Fig. 12). All populations had a negative population growth. Under the same scenario inbreeding depression also significantly increased the probability of extinction for all populations (Table 24).

Further analysis on all scenarios and all populations indicated that inbreeding depression consistently impacting annual population growth significantly in all populations (Mann-Whitnney U test, $p=0.019$) as well as the probability of extinction of Java sparrow populations within 100 years (Mann-Whitnney U test, $p = 0.024$).

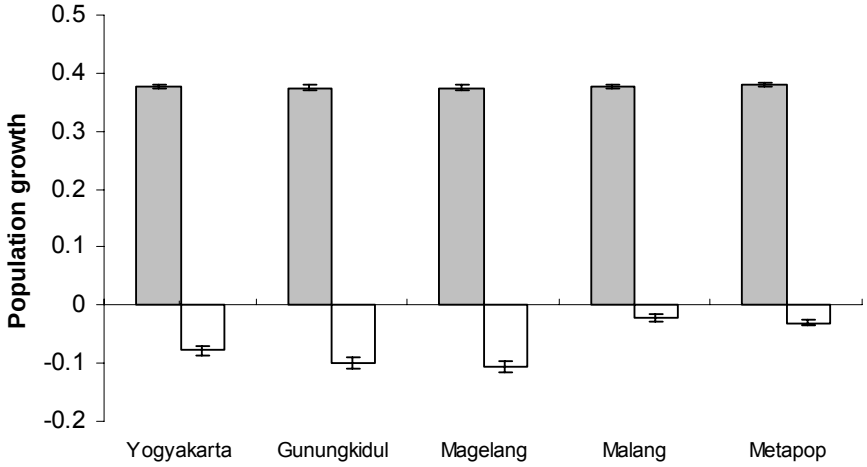


Figure 12. The impact of inbreeding depression (LE=12.3) on the population growth (±SE) of the population model under the baseline scenario on the remnant populations of Java sparrow. Grey bar = without inbreeding depression (LE=0), white bar = with inbreeding depression (LE=12.3)

6.4.3. Effect of trapping

Incorporating the current level of trapping in Yogyakarta into the model under the baseline scenario resulted in negative population growth in all remnant populations (Fig. 13) with a mean time to extinction between 14 to 34 years. Extinction times were shorter if dispersion was incorporated into the model (Table 25). This model assumed a constant proportional rate of harvesting every year during 100 years.

The sensitivity test on the level of trapping revealed that decreasing the level of trapping increased the population growth (Fig.13) and decreased the probability of extinction (Table 25). Decreasing the level of trapping to 30% decreased significantly the probability of extinction for larger population sizes (> 100) but not for smaller ones (n<40). Meanwhile the lower level of trapping (20%) had no impact on the probability of extinction for the larger population (>100) and less than 10% for the smaller population (Fig.14).

Table 24. The impact of inbreeding depression on the probability of extinction of the remnant populations of Java sparrow under six different scenarios
r: population growth; PE: probability to extinct; TE: time to extinct; LE= Lethal equivalent; NE=not extinct)

Scenario	Population		r	PE	MeanTE
A	Gunungkidul	LE(0)	0.375	0	NE
		LE(12.3)	-0.100	1	14.8
	Yogyakarta	LE(0)	0.377	0	NE
		LE(12.3)	-0.078	0.994	31.4
	Magelang	LE(0)	0.375	0.002	5.5
		LE(12.3)	-0.105	1	14.8
	Malang	LE(0)	0.376	0	NE
		LE(12.3)	-0.023	0.665	63
	Metapop	LE(0)	0.380	0	NE
		LE(12.3)	-0.030	0.661	64.2
B	Gunungkidul	LE(0)	0.176	0.016	12.3
		LE(12.3)	-0.139	1	14.5
	Yogyakarta	LE(0)	0.176	0.002	13.5
		LE(12.3)	-0.124	1	20.3
	Magelang	LE(0)	0.173	0.086	11.1
		LE(12.3)	-0.148	1	10.8
	Malang	LE(0)	0.178	0	NE
		LE(12.3)	-0.084	0.095	40.2
	Metapop	LE(0)	0.184	0	NE
		LE(12.3)	-0.096	1	41
C	Gunungkidul	LE(0)	-0.130	0.999	14.3
		LE(12.3)	-0.289	1	9.6
	Yogyakarta	LE(0)	-0.119	1	20.5
		LE(12.3)	-0.288	1	9.6
	Magelang	LE(0)	-0.135	1	10.9
		LE(12.3)	-0.286	1	6.4
	Malang	LE(0)	-0.109	0.999	31.4
		LE(12.3)	-0.255	1	13.4
	Metapop	LE(0)	-0.113	0.998	35.2
		LE(12.3)	-0.310	1	14
D	Gunungkidul	LE(0)	-0.035	0.846	29.1
		LE(12.3)	-0.205	1	9.7
	Yogyakarta	LE(0)	-0.025	0.717	40.4
		LE(12.3)	-0.194	1	13
	Magelang	LE(0)	-0.043	0.923	22.5
		LE(12.3)	-0.200	1	7.8
	Malang	LE(0)	-0.014	0.381	58.8
		LE(12.3)	-0.161	1	21.9
	Metapop	LE(0)	-0.010	0.23	67.8
		LE(12.3)	-0.182	1	22.4

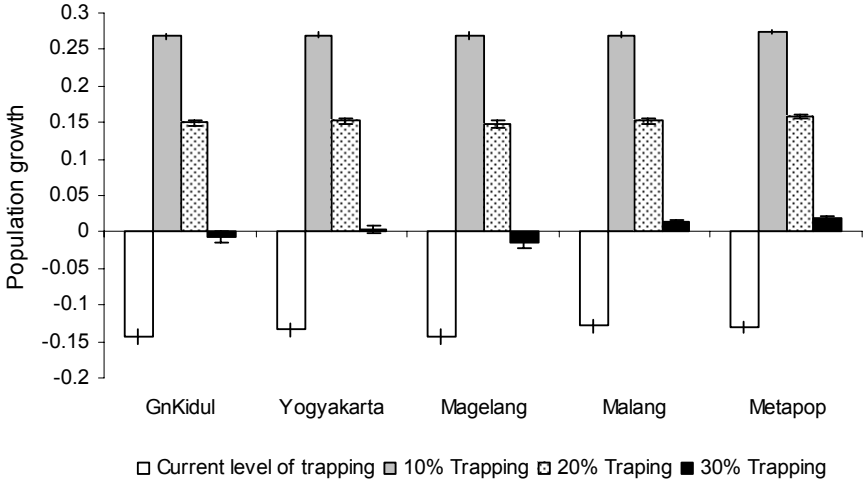


Figure 13. The impact of trapping on the population growth (±SE) of Java sparrow

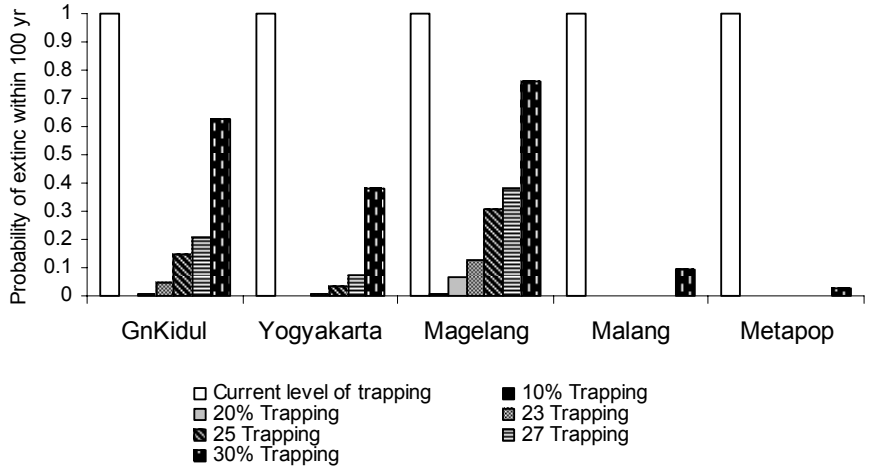


Figure 14. The impact of trapping on probability of extinction within 100 years

Table.25. Sensitivity analysis on of the level of trapping
(r: population growth; PE: probability to extinct; TE: time to extinct)

Population	Level of trapping	No Dispersion			With Dispersion		
		r	PE	MeanTE	r	PE	MeanTE
Gunungkidul	Current level	-0.143	1	16.9	-0.098	1	23.9
	10%	0.268	0.002	9.5	0.177	0	0
	20%	0.15	0.01	17.9	0.098	0	0
	30%	-0.007	0.624	31.7	0.006	0.35	60.3
Yogyakarta	Current level	-0.134	1	22.2	-0.147	1	20.2
	10%	0.27	0.001	6	0.109	0	0
	20%	0.153	0.001	15	0.04	0	27
	30%	0.004	0.381	43.9	-0.024	0.547	46.1
Magelang	Current level	-0.143	1	14	-0.178	1	11.1
	10%	0.269	0.006	9.8	0.026	0	14.2
	20%	0.148	0.065	13.8	0.014	0	18.4
	30%	-0.015	0.762	25.4	-0.023	0.65	17.4
Malang	Current level	-0.129	1	31	-0.5	1	8.5
	10%	0.27	0	0	-0.018	0	15.5
	20%	0.153	0	0	-0.024	0.007	12.5
	30%	0.013	0.091	57.1	-0.188	0.798	10.3
Metapop	Current level	-0.131	1	34.4	-0.172	1	26.3
	10%	0.274	0	0	0.126	0	0
	20%	0.157	0	0	0.065	0	0
	30%	0.018	0.024	69.3	-0.017	0.349	62.9

6.5. Discussion

Currently, Java sparrow numbers in the wild are very small and the species is highly susceptible to extinction. The results of the PVA analysis presented here suggest that under the baseline scenario the intrinsic rate of natural increase is about 0.37 for local populations and/or the larger metapopulation, meaning that, given these conditions, the populations would likely be able to recover. Incorporating inbreeding depression due to current small population sizes in the models decreased population growth but only significantly changed the probability of extinction within a 100 year period at very small population sizes (n <20, i.e. Magelang). However, impacts from other external deterministic factors, principally trapping, significantly

altered these findings. If trapping continues at current levels, the PVA analysis suggests that it is likely the Java sparrow will become extinct within one to three decades.

Sensitivity analysis indicated that modelled populations were most sensitive to changes in fecundity and mortality rates. A high rate of mortality or low fecundity in the baseline scenario caused negative population growth and resulted in a 98% probability of extinction. The higher mortality rates used in this analysis were calculated from a resighting formula derived from mark recapture data. Whether the cause of the low level of resightings was due to mortality or dispersal is unknown, making this a worst case scenario. More moderate levels of mortality changed population growth rates, but did not significantly alter the probability of extinction. A similar pattern was found for changes in the level of fecundity. This finding implies that if the fecundity is high (4.8 ± 1.5), the Java sparrow will not face a high extinction risk unless their mortality rate is high (>60% for adults, >53% for fledglings). However, if their fecundity is low (2.8 ± 1.9), low levels of mortality will already cause a high probability of extinction (>71%) in very small populations (n<40) and lower probability (23 – 38%) in larger populations (n>126).

Inbreeding depression is believed to have a significant impact on the long-term viability of a species (i.e. Frankham and Ralls 1998; Brook *et al.* 2002). Using a large value for lethal equivalent (LE=12.3), this study found that over the short-term (100 years) inbreeding depression significantly decreased the population growth in all populations and significantly impacted the probability of extinction of all remnant populations of Java sparrows. Hence, this finding highlights the potentially significant role of inbreeding depression on population viability for small populations, not only in the long-term, but also in the short-term. If this level of inbreeding depression occured in the Java sparrow, the remnant populations will become extinct within 15-64 years. To maintain the viability of these populations, in parallel with minimizing the main threat (i.e trapping, see below), an augmentation program is suggested for these small populations. Given that mtDNA variation in the introduced population from Kalimantan is not significantly different from that found in natural populations and the birds are relatively more abundant at this location, this population could be considered as a potential source of birds for an augmentation program.

The current level of trapping of Java sparrows is very high. Even using the average level of observed adult harvesting (38%), population modelling suggested that the Java sparrow was at an extremely high risk of extinction over the short term. This finding highlights the urgent need to minimize the level of trapping, rather than concentrating on other factors which may also affect the persistence of the bird, such as nesting or foraging habitat availability. However, the trapping (and trading) of Java sparrow will likely continue because of the deep cultural traditions of bird keeping in Indonesia (Jepson and Ladle 2005) and other socio-cultural uses of Java sparrows. The modelling of some declining species suggests that harvesting a declining species using a particular harvesting strategy can maintain both sustainable use and minimum viable populations (Lande *et al.* 1997; Tufto *et al.* 1999; Nilsson 2004). A similar approach applied to the harvesting of Java sparrows may suggest trapping thresholds and limits within which harvesting is possible.

Under the baseline scenario of this model, trapping rates of 30% of population size were sustainable for the larger metapopulation, but not within each of the local populations. To meet the IUCN's criteria for Vulnerable species, the probability of extinction is at least 10% within the next 100 years, hence the threshold trapping on local population to maintain this level of viability was around 23% of current population size. Given that this model is based on the best scenario (high fecundity, low mortality and no inbreeding), and that trapping demand already appears to exceed this level, extreme caution and intensive monitoring would need ned to be established if this level of harvesting was sustainable. A substantially lower harvesting threshold would be required to minimize the extinction risk for the Java sparrow, particularly during re-establishment.

The reliability of using PVA as a risk analysis for endangered species is often discussed, particularly due to the limited availability of reliable data to be used for modelling. This is also the case for the Java sparrow. However, in light of the fact that PVA is also considered an ongoing process (Morris and Doak 2002) that can be used to identify the focus of further studies, the findings from this PVA highlight the critical need for further detailed field studies aimed at establishing robust estimates of demographic parameters and other breeding characteristics.

In summary this study found that, under the best case PVA scenario used, Java sparrow populations would be able to recover from their current vulnerable

status. Sensitivity analysis revealed that the models were most sensitive to changes in mortality and fecundity and highlighted the need for further field studies on these parameters to gain a more realistic assessment of the fate of the Java sparrow. It was also revealed that current levels of trapping are not sustainable and if they continue the Java sparrow is likely to become extinct within a 5 to 35 year time frame. This finding emphasizes the critical short-term need to formulate a trapping/harvesting strategy to minimize the extinction risk. Given that eradication of trapping seems unlikely, at least in the short-term, working thresholds need to be established as a short term management option for the Java sparrow and their potential impact monitored so that effective and sustainable management strategies can be developed over the longer term.

CHAPTER 7. GENERAL DISCUSSION

This study combined field studies, molecular analyses and population modelling to establish the current population status, level of continued threat, historic phylogeography, contemporary connectivity among remnant populations, genetic diversity, and population viability of the endangered Java sparrow. The findings confirmed that remnant populations of the Java sparrow are small and severely fragmented, and that the principal deterministic threat (i.e. trapping) was ongoing at high levels. Genetic diversity was relatively low, but this was not only as a direct result of recent population declines. The results of the PVA suggest that, under an optimistic baseline scenario, the populations would likely be able to recover. In spite of this, incorporating inbreeding depression due to current small population sizes and other external deterministic factors (i.e trapping) suggests that it is likely the Java sparrow will become extinct within one to three decades. This chapter will further discuss these findings and their implications for the conservation of this vulnerable species.

7.1. Current distribution and abundance

Previous studies used limited data to suggest that populations of Java sparrow were small and fragmented throughout the species natural range (Balen 1997; Laudisensius *et al.* 2000; Muchtar and Nurwatha 2001). Using more robust census methods, the results of this study confirmed these previous reports. The total population of Java sparrows in Central and East Java were no greater than ~1000 individuals. Among the sites studied, no sub-population was estimated to contain more than 300 individuals. If this pattern is consistent across other regions, then Java sparrow populations within the species natural range remain very small and fragmented. For this reason I believe that the total Java sparrow population in its natural range is likely to be at the lower end of the range from 2500 – 10000 individuals used to classify the species as 'Vulnerable' (IUCN 2006).

Further population studies in other regions are necessary to confirm these predictions outside of the areas studied here. Given its rarity and the wide extent of its major habitat (i.e. urban and cultivated areas), identifying and locating the staging sites of nesting Java sparrows is the key to getting more effective and reliable estimates of Java sparrow population changes through time. Failure to locate all staging sites, however, will produce an underestimated of population size for studied areas. This study found that incorporating bird market and trapper surveys into the research design provided a significant contribution to identifying the most recent occurrence of Java sparrows.

7.2. Population fragmentation and genetic differentiation

This study also found that the remnant populations of the Java sparrow were severely fragmented. However, molecular analysis using two different DNA marker types produced inconsistent results. Based on mtDNA control region sequences, currently the Java sparrow is likely a single panmictic population throughout its native range. However, this finding must be interpreted with caution. Significant evidence of a recent population expansion means that the assumptions of standard AMOVA may be violated. If so, then much of the mtDNA signal in the data could represent historic rather than contemporary associations among populations. Hence, to reveal the current connectivity among remnant populations, I conducted multi-locus microsatellite genotyping.

In contrast to the mtDNA data, microsatellite genotyping indicated significant structuring of contemporary Java sparrow populations, but lack of differentiation among historical samples, which were collected 50-60 years ago before any perceived population decline. This finding suggests that limited connectivity among remnant populations is a result of recent population declines and fragmentation due to human induced impacts.

The lack of mtDNA differentiation and limited but significant microsatellite differentiation also suggests that all Java sparrow populations are part of a single Evolutionarily Significant Unit (ESU) and Management Unit (MU) - as define by Moritz (1994). However, this findings must also be interpreted with caution, since only five microsatellite loci were used and sample sizes for some populations were

limited (i.e. Magelang, Gunungkidul, Bali and all historical samples). Identification of ESUs and MUs is sensitive to error due to the small number of samples (Moritz 1994).

This finding suggests that, for genetic management of the Java sparrow, the remnant populations should not be managed as separate isolated populations. To enhance the genetic diversity of the remnant populations, the introduced populations in Kalimantan may offer a source of genetically similar birds for use in a reintroduction or augmentation program. However, to develop a sound conservation strategy for this species, it is important to take into account the concept of "ecological exchangeability" (Crandall *et al.* 2000). For this purpose we need studies on behaviour, life history, morphology, and environmental interactions to get a more meaningful assessment of biologically relevant differentiation among remnant Java sparrow populations.

7.3. Limited genetic variation

Theoretically, genetic diversity is related to population size. This theory is based on two assumptions: 1) that most genetic diversity is neutral in small populations, and 2) the existing population sizes reflect historic effective population sizes (Frankham *et al.* 2002). For this reason many conservation biologists believe that small populations of threatened species should have low levels of genetic variation (e.g. Frankham 1995). A review study on a wide range of plant and animal taxa provides general support for this relationship (Frankham 1996), even though empirical evidence from natural populations does not always show such relationships (e.g. Madsen *et al.* 2000; Nichols *et al.* 2001),.

In my study, molecular analysis using mtDNA control region and microsatellite markers revealed that genetic diversity of the Java sparrow was relatively low, and in the mid-range of genetic diversity for endangered finch species. However, the recent rapid population decline due to trapping appears to have had little significant impact on either mtDNA or microsatellite variation, suggesting that the low genetic variation in the Java sparrow was likely due to historical demographic processes. Analysis of mtDNA sequences showed that Java sparrow populations experienced a bottleneck and subsequent expansion during the last glacial maxima of the Pleistocene, or more recently in the Holocene, depending on

the evolution rate used in the analysis. Analyses per island draw parallel results to Hewitt's observation (Hewitt 1999) that loss of genetic variation can occur during expansion. In the case of the Java sparrow, populations in Java have more genetic variation than those of Madura and Bali suggesting that the Java sparrow expanded from Java to these other islands.

7.4. Current and Potential Threats

Previously, it has been suggested that the major threat to Java sparrow population viability is trapping and trade (Balen 1997; Shanaz *et al.* 2000; BirdLifeInternational 2001; 2006). This study confirmed that this major threat continues at a high level. An interesting finding was that both captive bred and wild birds imported from introduced populations outside Java have major roles in fulfilling market demand. Even so, it is unlikely that the importation from outside Java currently reduces the level of trapping of wild birds sufficiently to allow wild populations inside Java to remain viable, particularly for remnant populations in the central region.

This finding implies that minimizing further trapping should be the first priority of any future management plan for this declining species. The protection of roosting and nesting sites from trapping is the first step in any effort to save the Java sparrow. The Malang and Prambanan birds, though not in a nature reserve, profit by indirect protection from the sites' authorities. Meanwhile, trapping occurs in associated feeding areas (i.e., paddy fields), where Java sparrows are generally considered a pest. Although these areas are on private land, the owners or people living in the surrounding areas do not prevent bird trappers depleting local populations. This was also true for the Jothak population, which experienced intense exploitation at the nesting site. For this reason it is very important to involve local people in conservation initiatives for Java sparrow. This may include encouraging the local people to initiate small scale breeding projects. It is likely that existing commercial breeding already significantly mitigates the extraction of birds from wild populations. Further promotion of commercially-bred alternative birds has been suggested as an effective and popular solution to the high demand for birds in Indonesia (Jepson and Ladle 2005).

In addition to strengthening the above initiatives, the Java sparrow should also be listed as a protected species. It is surprising that the Java sparrow has not appeared on the list of Protected Species in Indonesia. However, local conservation initiatives have already been taken by local government (e.g. Surabaya) and local community groups (e.g. *awig-awig* in Bali). Other local governments and/or communities should follow these initiatives. Such initiatives are very important, as most of the remnant populations are not in nature reserves or other protected areas in Indonesia.

Other factors have been suggested as threats for the Java sparrow, i.e. intensive used of pesticide, and competition with the Eurasian Tree sparrow (Balen 1997; Shanaz *et al.* 2000; BirdLifeInternational 2001; 2006). So far, however, only the last factor has been studied systematically. These studies found that competition between the Java sparrow and Eurasian tree sparrow was not consistently present. Evidence from Sukawati (Bali), Sukabumi (West Java) and Malang (East Java) clearly suggests the occurrence of competition between the two species (Muchtar and Nurwatha 2001), but not in the Prambanan temples complex and Malang (*pers. obs.)* in which the two species co-exist. Therefore, further studies on these aspects are suggested to gain a more comprehensive understanding of this factor as a mechanism of decline.

7.5. Population viability

The results of the PVA (Chapter 6) suggest that under the baseline scenario Java sparrow populations would likely be able to recover. In spite of this, incorporating inbreeding depression due to current small population sizes and other external deterministic factors (i.e. trapping), suggests that it is likely the Java sparrow would become extinct within one to three decades. If the latter is true, the critical short-term need is to prevent trapping in order to conserve the Java sparrow. Unfortunately, the cessation of trapping or the use of alternative protection initiatives (see section 7.4) seems unlikely in the short term. Therefore, at least in the short-term, working thresholds need to be established as a management options for the Java sparrow and their potential impact monitored, so that effective and sustainable management strategies can be developed over the longer term.

In order to support the recovery of remnant populations, enhancing population size is another alternative. Population augmentation may include supportive breeding (Ryman and Laikre 1991) and/or restocking (Primack 2002). Supportive breeding involves removing a fraction of individuals from wild populations into captive breeding programs and re-introducing the offspring into native remnant populations (Ryman and Laikre 1991), (e.g. Magelang, Yogyakarta). However, supportive breeding may elevate rates of inbreeding and genetic drift in wild populations (Hogan *et al.* 2004). Alternatively, restocking could be undertaken using individuals taken from established but introduced populations (e.g. from Kalimantan), which are genetically (mtDNA) similar (Chapter 3). Even so, care should be taken, since introducing new individual birds into native populations can raise other problems such as introducing disease. Moreover, to establish an effective augmentation program, other factors such as ecological and behavioural aspects of the birds' life history also need to be considered. These alternative programs should be implemented in parallel with a harvesting management program, otherwise population enhancement would not be effective (Collins *et al.* 1998).

Given the limited availability of reliable data used in this PVA and in light of the fact that PVA is also considered an ongoing process (Morris and Doak 2002) used to identify the focus of further studies, the findings from this PVA highlight the critical need for further detailed field studies aimed at establishing robust estimates of demographic parameters and other breeding characteristics. By doing so, a more realistic assessment on the fate of the Java sparrow could be achieved.

7.6. Conservation Status

The current conservation status of the Java sparrow is Vulnerable (BirdLifeInternational 2001). Eventhough the best available data were poor (BirdLifeInternational 2006), it is believed to meet the criteria of A1a,c,d; A2c,d; C1 (IUCN 2001). This means that the Java sparrow population has declined by ≥50% over the last 10 years and the causes of the reduction are clearly reversible and understood and have ceased (criteria A1), and/or there has been a population size reduction of ≥30% over the last 10 years where the reduction or its causes may not have ceased or may not be understood or may not be reversible (criteria A2). The

population size of the Java sparrow was estimated at less than 10,000 mature individuals, with an estimated decline of at least 10% within 10 years (criteria C1).

In contrast, the findings of this study, suggest that the Java sparrow meets the criteria to be transferred from Vulnerable to Endangered. The reasons for this proposal are as follows:

- An observed population size reduction of the biggest populations in Prambanan and Malang of more than 50% over the last 10 years. It is believed that this same rate of decline has also been experienced by the other remnant populations. Bird trapping, the main cause of the reduction, is continuing (Chapter 5). These findings meet criteria of A2a,b,d.
- The results of the PVA (Chapter 6) indicate that if the current level of trapping continues the probability of the Java sparrow becoming extinct within the next 100 years is 100% for all remnant populations with the times to extinction (TE) being less than 20 years for all populations. These findings meet criteria E of Endangered species: *quantitative analysis showing the probability of extinction is at least 20% within 20 years or five generations, whichever is the longer* (IUCN 2001).

7.7. Conclusion

This study aimed to asses the current population distribution, abundance and existing threats to the Java sparrow, identify the effect of historic demographic processes on genetic diversity and current connectivity among remnant populations and to analyse the population viability of the endangered Java sparrow under current trapping regimes. The findings of this study can be summarized as follow:

1. Remnant populations of Java sparrow in Central and East Java are small and fragmented. Total population estimates of Java sparrows in Central and East Java were no greater than ~1000 individuals. If this pattern is consistent across other regions then Java sparrow populations within the species natural range remain very small and fragmented. For this reason I believe that the total Java sparrow population in its natural range is likely to be at the lower end of the range from 2500 – 10000 individuals. However, analysis on population reduction and

quantitative analysis (PVA) suggest that the Java sparrow should be transferred from Vulnerable to Endangered species (A2a,b,d; E).

2. The major threats, trapping and trade, continue. This is particularly true for Central Java, but less so for East Java. Captive bred and imported wild birds from introduced populations have a major role in fulfilling market demand at certain times of year. Encouraging captive breeding could reduce the threat of trapping to the natural populations.

3. Molecular analysis using an mtDNA control region revealed that the Java sparrow populations experienced a demographic bottleneck and subsequent expansion during the last Pleistocene glacial maxima or more recently in the Holocene, depending on the molecular mutation rate used in analysis. No mtDNA differentiation was observed between contemporary populations suggesting Java sparrow is a single population and might be considered as a single Evolutionarily Significant Unit.

4. In contrast, the results of multi-locus microsatellite genotyping indicated limited but significant structuring of contemporary remnant populations but no structuring among historical samples, suggesting that recent human induced fragmentation and population decline are responsible.

5. Genetic variability of the Java sparrow in both mtDNA and microsatllite markers, is relatively low, but this does not appear to be the result of recent population declines.

6. A population viability analysis using stochastic modelling applied in VORTEX revealed that under the basic scenario the Java sparrow will survive over the next 100 years. However, if the current level of trapping continues, the Java sparrow will be extinct within one to three decades. This analysis emphasizes the need for further research on demographic parameters and breeding biology to gain a more realistic prediction of the population viability of the Java sparrow.

7. The critical short-term management need for this species is to formulate a trapping/harvesting strategy and establish working thresholds to minimize the extinction risk. This strategy can then be used as a basis for more effective and sustainable management strategies over the longer term.

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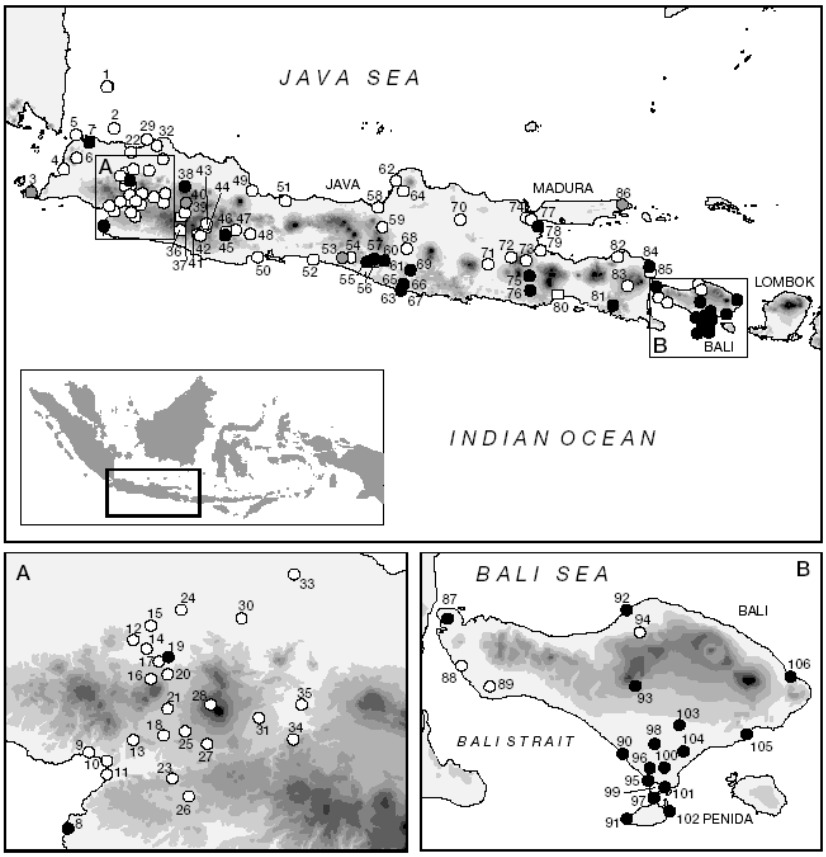
APPENDICES

Appendix 1. The distribution of locality records of Java sparrow in its natural range from 1881 till 2000 (BirdLifeInternational 2006)



The distribution of Java Sparrow *Padda oryzivora*: (1) Pulau Jaga Utara; (2) Pulau Payung; (3) Ujung Kulon National Park; (4) Labuan; (5) Lebak Gede; (6) Ujungtebu; (7) Pulau Dua; (8) Cikepuh; (9) Cimaja; (10) Pelabuhanratu; (11) Cidadap; (12) Gobang; (13) Gunung Masigit; (14) Pasir Gaok; (15) Jampang; (16) Gadog; (17) Ciomas; (18) Sukamaju; (19) Bogor; (20) Cijeruk; (21) Cicurug; (22) Jakarta; (23) Pasir Kananga; (24) Depok; (25) Caringin; (26) Cibening; (27) Sukabumi; (28) Cimungkat; (29) Muara Bungin; (30) Cibarusa; (31) Cianjur; (32) Batujaya; (33) Kedunggede; (34) Cihea; (35) Cibungur; (36) Ciheulang; (37) Cikalong; (38) Curug Cijalu; (39) Banjaran; (40) Bandung; (41) Gunung Papandayan; (42) Cikajang; (43) Garut; (44) Ngamplang; (45) Tanjung; (46) Marai; (47) Carui; (48) Banjar; (49) Cirebon; (50) Pangandaran; (51) Brebes; (52) Karangbolong; (53) Kutoarjo; (54) Purworejo; (55) Godean; (56) Yogyakarta; (57) Depok; (58) Semarang; (59) Gedangan; (60) Kalasan Temple; (61) Prambanan; (62) Jepara; (63) Gunung Kidul; (64) Cilegong; (65) Paliyan; (66) Panggang; (67) Tepus; (68) Solo; (69) Gajah Mungkur reservoir; (70) Padangan; (71) Kediri; (72) Wonosalem; (73) Gunung Arjuno; (74) Manyar; (75) Malang; (76) Gondang Legi; (77) Gresik; (78) Surabaya; (79) Bangil; (80) Dampar; (81) Meru Betiri National Park; (82) Klatakan; (83) Gunung Raung; (84) Baluran National Park; (85) Bajulmati; (86) Batang Batang; (87) Bali Barat National Park; (88) Candikusuma; (89) Negara; (90) Tanahlot; (91) Ulu Watu; (92) Buleleng; (93) Wangaya Gede; (94) Gitgit; (95) Petitenget; (96) Kerobokan; (97) Suwung; (98) Abian Base; (99) Kuta; (100) Denpasar; (101) Pesanggaran; (102) Nusa Dua; (103) Ubud; (104) Sukawati; (105) Padangbai; (106) Amed.

○ Historical (pre-1950) ◎ Fairly recent (1950–1979) ● Recent (1980–present) □ Undated

Appendix 2. Population of Java sparrow from 1999 – 2001

Location	Habitat	Number of Java sparrow	Reference
West Java:			
Citiis	Hl, Rf, Wl edge	6	1
Ciburial	Hl, Rf, Gd	6+9	1
Curug Cijalu	Te, Af, Gd	5	1
Central Java:			
Magelang	St, Rf	16-19	4
Depok, Nologaten	Rf	3 –6	2
Kledokan	Rf,		2
Babarsari	Wl		2
Prambanan Temples	Tp	1; 23, 33-68	1; 2; 5
Kalasan Temple	Tp	5	2
Sari Temple	Tp	7	2
Barong Temple	Tp	3	2
Ijo Temple	Tp	17	2
Sidoarum	Rf	5	2
Sendang Sari	Rf	1	2
Song Dawung	Kr, df	17	2
Putat, Song Banyu	Rf	3	2
Purwodadi, Tepus	Rf	3	2
Kanogoro, Paliyan	Rf	2	2
Girikerto, Pangang	Rf	6	2
Gelatik Is	Is, Gr	3	2
Gua Slawu, Melikan	Kr	1	2
East Java:			
Kantor Bupati Malang	Oc, USt	19	1
Rowopulo, Gumukmas Jember	?	2	1
Bali:			1
Sukawati Gianyar	St, Tp	6 - 7	1
Gg Garuda Ubung	St	4	1
Abian base, Mengwi	St, Rf	17+11	1
Kerobokan	St, Rf	6+6	1
Tanah Lot	St, Rf	3	1
Tuwed	St, Mg	1	1
Dauh Puri Raja	USt	2+2	1
Kampung Bugis, Buleleng	CSt	1+2	1
Kampus UNUD, Bukit Jimbaran	Gl	1	1

(continued)

Location	Habitat	Number of Java sparrow	Reference
Kampus Unud	St	2	1
Gerogak	Rf	16	3
Pelapuan	Rf	18	3
Bakung	Rf	13	3
Candikusuma	Rf	16	3
Tibubiyu	Rf	30	3
Sanur	Rf	24	3
Tamanbali	Rf	28	3
Sidemen	Rf	18	3

Note: Habitat – Hl: hamlet, Rf –Ricefield, WL – Woodland, Gd –Garden, Te-tea plantation, Al –Alang-alang field, St –stlement (U-urban, C-coastal), Tp – temple, Kr – karst area, Mg –Mangrove, Gl – grassland, Oc – office complex; Reference: 1 - (Muchtar and Nurwatha 2001), 2 - (Laudisensius *et al.* 2000), 3 -(Surata 2000), 4 -(Anonymous 2003), 5-

Appendix 3. Sample loan from museum used for this study (MZB: Museeum Zoological Bogor (Indonesia); RNHM: Raffles Natural History Museum (Singapore)

No	Collection Catalouge	Collection Date	Collection Site	Sex	Museum deposit
1.	19764	21.1.38	Jakarta	f	MZB
2.	19769	25.2.36	Jakarta	f	MZB
3.	19772	26.1.38	Jakarta	m	MZB
4.	19778	17.12.37	Jakarta	m	MZB
5.	19460	17.12.37	Jakarta	m	MZB
6.	19777	17.12.37	Jakarta	f	MZB
7.	19756	31.12.37	Jakarta	m	MZB
8.	19758	19.12.37	Jakarta	f	MZB
9.	19757	1.12.37	Jakarta	f	MZB
10.	19761	19.12.37	Jakarta	m	MZB
11.	19864	26.8.39	Semarang	F	MZB
12.	19854	24.8.39	Semarang	M	MZB
13.	19832	26.8.39	Semarang	M	MZB
14.	18774	18.11.32	Semarang	M	MZB
15.	13814	1.8.31	Semarang	M	MZB
16.	19850	24.8.39	Semarang	F	MZB
17.	19868	12.10.39	Semarang	F	MZB
18.	19871	26.8.39	Semarang	F	MZB
19.	19851	12.10.39	Semarang	?	MZB
20.	19843	24.8.38	Semarang	M	MZB
21.	19929	30.9.39	Solo	F	MZB
22.	19901	30.9.39	Solo	F	MZB
23.	19899	30.9.39	Solo	F	MZB
24.	19915	30.9.39	Solo	M	MZB
25.	19884	5.10.39	Solo	F	MZB
26.	19880	30.9.39	Solo	F	MZB
27.	19904	3.10.39	Solo	M	MZB
28.	19930	5.10.39	Solo	F	MZB
29.	19896	30.9.39	Solo	M	MZB
30.	19913	27.10.37	Solo	?	MZB
31.	19796	7.10.39	Surabaya	M	MZB
32.	19818	7.10.39	Surabaya	F	MZB
33.	19790	3.10.39	Surabaya	F	MZB
34.	19781	7.10.39	Surabaya	M	MZB
35.	19810	21.10.39	Surabaya	M	MZB
36.	19786	28.10.39	Surabaya	M	MZB
37.	19785	7.10.39	Surabaya	F	MZB
38.	19806	21.10.39	Surabaya	F	MZB
39.	19803	28.9.39	Surabaya	M	MZB
40.	19808	2.10.39	Surabaya	F	MZB

(continued)

No	Collection Catalouge	Collection Date	Collection Site	Sex	Museum deposit
41.	K.17	13.1.41	Tikus Island, CocosKeeling	M	RNHM
42.	K.26	10.2.41	Tikus Island, CocosKeeling	M	RNHM
43.	K.30	3.3.41	Tikus Island, CocosKeeling	F	RNHM
44.	K.29	3.3.41	Tikus Island, CocosKeeling	M	RNHM
45.	K.21	13.1.41	Tikus Island, CocosKeeling	M	RNHM
46.	K.24	13.1.41	Tikus Island, CocosKeeling	F	RNHM
47.	K.18	13.1.41	Tikus Island, CocosKeeling	M	RNHM
48.	K.20	13.1.41	Tikus Island, CocosKeeling	M	RNHM
49.	K.19	13.1.41	Tikus Island, CocosKeeling	F	RNHM
50.	K.27	10.2.41	Tikus Island, CocosKeeling	F	RNHM
51.	K.23	13.1.41	Tikus Island, CocosKeeling	F	RNHM
52.	K.25	13.1.41	Tikus Island, CocosKeeling	M	RNHM
53.	K.28	3.3.41	Tikus Island, CocosKeeling	F	RNHM