# Evidence of genetic connectivity between fragmented pig populations in a tropical urban city-state

Joshua J-M. Koh*, Frank E. Rheindt, Elize Y.X. Ng & Edward L. Webb

**Abstract.** Forest fragmentation as a result of urbanisation can adversely affect gene flow between wildlife populations. Although gene flow among fragmented populations has been investigated for many species, there has been little research into the effects of urbanisation on gene flow in large mammals. Singapore is a small, densely urbanised tropical city-state where more than 99% of its original forest has been cleared. The wild pig is currently the largest native terrestrial mammal in Singapore and has important roles in maintaining plant diversity and seed dispersal. Pigs are widespread and present in the Central Catchment Nature Reserve (CCNR) as well as other forest patches in Singapore. Due to the extent of urbanisation, it is unclear if genetic connectivity exists between the CCNR and other forest fragments. In this study, genome libraries from 48 samples of pig blood were collected from two sites: 1) the CCNR and 2) forest fragments in the northeast of Singapore. Genome-wide SNP loci were used to understand the population genomics of the two pig populations. The results indicated that the pig populations formed two distinct genetic clusters that did not align with the respective sites. Both sites included individuals with signatures from both genetic clusters, although with different frequencies. This suggests that pigs can move across heavily urbanised landscapes. Future studies should involve long term collaring studies to identify specific corridors used by the pigs for dispersal, and a larger population genomic assessment to understand pig dispersal and gene flow to and from forest patches other than the CCNR and the Northeast.

**Key words.** Fragmentation, gene flow, population genetics, *Sus scrofa*, urbanisation

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## INTRODUCTION

The expansion and intensification of agricultural and urban areas is one of the major causes of deforestation and habitat fragmentation (Andrén, 1994). Globally, more than half of the mixed and temperate broadleaf forest biome, and almost a quarter of the tropical rainforest biome, have been fragmented or removed by humans (Wade et al., 2003). Habitat fragmentation can alter spatial patterns that affect wildlife biodiversity and habitat quality (Gascon & Lovejoy, 1998; Jules et al., 1999; Kurki et al., 2000). One particular consequence of habitat fragmentation is the creation of barriers that reduce opportunities for animals to disperse (Arnold et al., 1993; Diffendorfer et al., 1995). This decrease in connectivity can constrain gene flow between fragmented populations, resulting in the reduction of genetic diversity and the loss of adaptive potential, inbreeding depression and the accumulation of deleterious mutations within fragmented populations (Slatkin, 1987; Allendorf & Lundquist, 2003; Keyghobadi, 2007).

Gene flow among fragmented populations has been investigated for many large mammals such as brown bears (Waits et al., 2000), common wallaroos (Arnold et al., 1993), European roe deer (Coulon et al., 2004), polar bears (Paetkau et al., 1995), red deer (Pérez-espona et al., 2008), western grey kangaroos, (Neaves et al., 2009), and desert bighorn sheep (Epps et al., 2005). However, the populations in these studies were fragmented by geographical features such as mountains and water bodies, agricultural areas or man-made structures such as aqueducts and highways. Until now, there has been little research into the effects of urbanisation on gene flow in large mammals (Epps et al., 2005; Frantz et al., 2012).

With the advent of novel high-throughput sequencing (HTS) technologies, the analysis of thousands of genome-wide markers has become much more affordable and accessible for both model and non-model organisms (Wagner et al., 2013). Based on their abundance throughout the genome and ease of modelling, single nucleotide polymorphisms (SNPs) are increasingly being used for population genomic analyses (Brumfield et al., 2003; Morin et al., 2004; Ng et al., 2017). Unlike traditional markers such as mitochondrial DNA and microsatellites, the abundance of SNPs in the genome can reveal genomic information at a higher resolution (Kjeldsen et al., 2016; Wagner et al., 2013).

The wild pig, *Sus scrofa*, is a large mammal that has a wide native distribution in most of Eurasia and North Africa

Department of Biological Science, National University of Singapore, 14 Science Drive 4, Singapore 117543; Email: j.ignatiuskoh@gmail.com (*corresponding author)
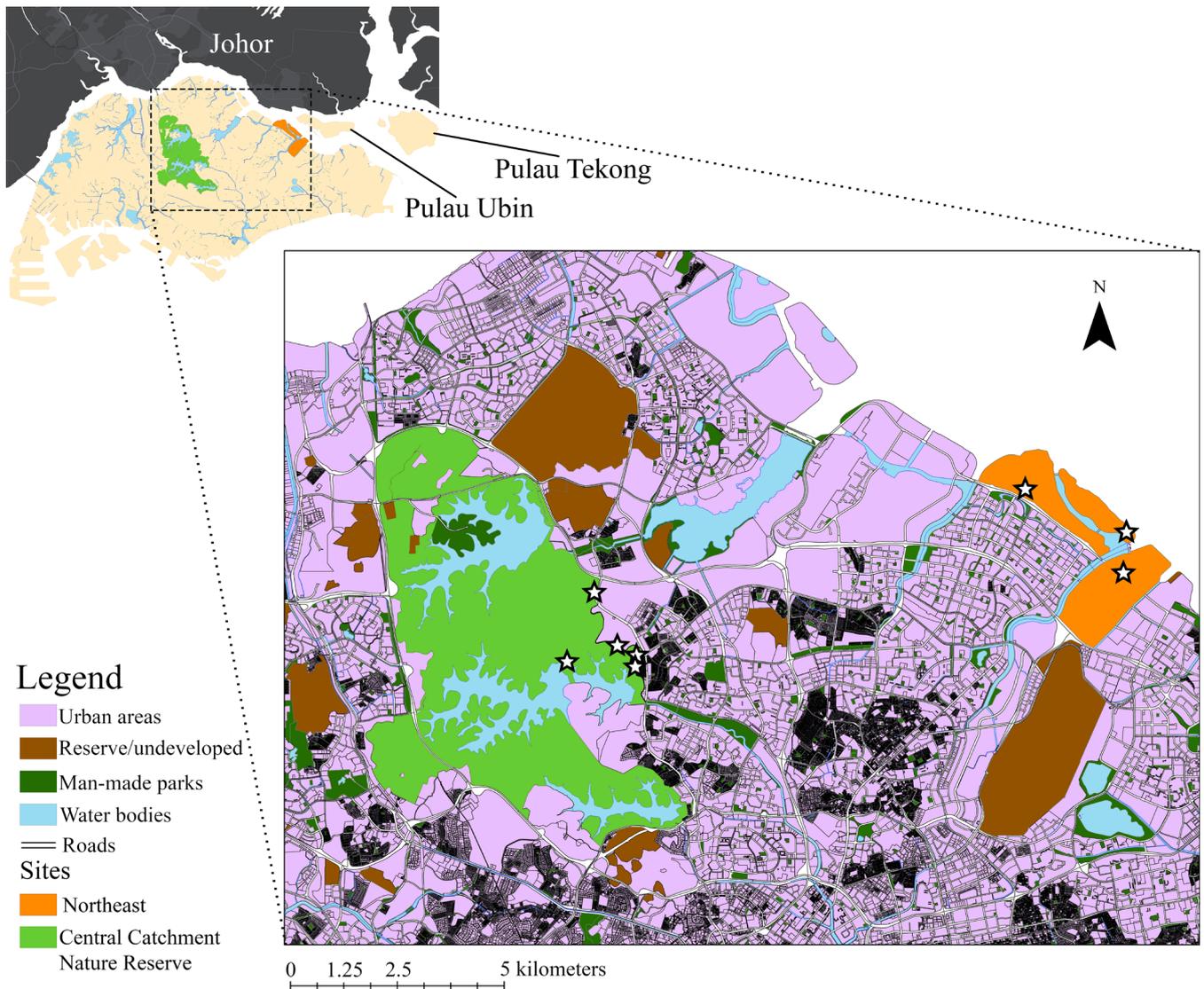
Fig. 1. Map of Singapore, in relation to Johor (Malaysia), showing various land-use types and the study sites (Central Catchment Nature Reserve, Northeast). Offshore islands Pulau Tekong and Pulau Ubin where pigs are also present are labelled. White stars indicate locations of cage traps.

and was subsequently introduced into other parts of the world by early explorers (Barrios-Garcia & Ballari, 2012). Because of their destructive behavior, pigs can heavily impact the environment and are considered ecosystem engineers (Crooks, 2002) that are important in maintaining plant diversity (Kotanen, 1995; Welander, 1995; Arrington et al., 1999), seed dispersal and colonisation (Matías et al., 2010). Additionally, wild pigs are generalists and can exploit the contact zones between natural habitats and urban areas (Radeloff et al., 2005; Cahill et al., 2012). This often results in conflicts between wild pigs and humans (Loker & Decker, 1998) and can range in severity, from damaged parks and backyards, to traffic accidents and attacks on humans and companion animals (Kotulski & König, 2008).

Singapore is a small (~700 km²), densely urbanised island city-state, with approximately 5.61 million human residents (Singapore Department of Statistics, 2018), located south of the southernmost tip of peninsular Malaysia (1°17′N, 103°50′E). Since the 1870s, more than 99% of the original forest in Singapore has been cleared (Corlett, 1992; Turner

et al., 1997). Today, 22.47% (163.07 km²) of Singapore's land area is forested, of which 11.13 km² is primary and old secondary forest, and 142.88 km² is young secondary forest comprising mostly of abandoned plantations that are both floristically and structurally much simpler than the original forest (Corlett, 1992; Yee et al., 2011). The remnant primary and old secondary forests are found exclusively in the largest reserve in Singapore, the Central Catchment Nature Reserve (CCNR) that covers approximately 32 km².

Although previously extirpated on the main island of Singapore, the wild pig is now widespread and has apparent strongholds in the CCNR, a group of forest fragments in the northeast of Singapore (Fig. 1) and forest fragments in western Singapore (Yong et al., 2010). Owing to the local extinction of its natural predators (*Panthera tigris tigris* and *Panthera pardus delacouri*), the pig is not naturally regulated by predators and is currently the largest native terrestrial mammal in Singapore (Corlett, 1992; Brook et al., 2003). It is unclear when wild pigs were extirpated on the main island of Singapore and when or how they recolonised it.

One hypothesis is that wild pigs were never locally extinct (Harrison, 1974) and escaped/were released from pig farms that existed in the northeastern side of Singapore's main island until 1990 (Taiganides, 1992). Because pigs are capable of swimming across 9 km of sea (Wallace, 1895), another hypothesis is that wild pigs swam across the narrow Straits of Johor from Pulau Ubin, Pulau Tekong, or Johor (Fig. 1) and established populations in forest fragments along the coasts of Singapore's main island.

Currently no studies have investigated how and when wild pigs recolonised Singapore's main island or if gene flow exists between the fragmented pig populations on Singapore's main island or between Singapore's main island and Malaysia/ Singapore's offshore islands. In this study, genome-wide SNPs were used to understand gene flow and connectivity between two wild pig (*Sus scrofa*) populations on the small tropical urbanised island of Singapore.

## MATERIAL AND METHODS

**Sample collection.** Due to limitations of the research permit, blood samples were only collected in the CCNR and the Northeast. A total of 48 blood samples were collected from individuals between June and September 2016. Of the 48 blood samples, 34 were collected from individuals caught on the eastern side of the CCNR, and the remaining 14 from the Northeast were donated by the Agri-Food and Veterinary Authority of Singapore (Fig. 1, Table 1). Individuals in the CCNR were caught using baited cage traps, which consisted of five to six modular steel panels and one trap door. Each steel panel was 2 m wide and 1.5 m tall and the trap door would fall when an animal enters the cage trap, triggering the release mechanism by pulling on a bait-filled mesh box. Cage traps were checked every weekday morning and bait was replenished every day. To prevent injury to the animals, the interior of the cage traps was lined with plastic barrels and the exterior was lined with an opaque shroud. Cage traps were only deployed on the eastern side of the CCNR as the pig population in the CCNR was most dense in that area (Koh, 2018).

Individuals that weighed more than 25 kg were too large to safely restrain and were sedated with a blow-pipe by a licensed veterinarian on-site. The veterinarian collected 3 ml of blood from the trapped individuals intravenously, introduced immediately into a falcon tube filled with 45 ml of 95% ethanol and mixed thoroughly. Trapped individuals were then tagged with ear tags, to prevent re-sampling, and released safely. Following blood collection, the falcon tubes were transported to the laboratory where they were stored at −20°C.

**DNA extraction and ddRADSeq library preparation.** DNA extraction was carried out with a DNEasy Blood & Tissue Kit (Qiagen) using a modified version of the manufacturer's protocol for blood. To maximise DNA yield, the samples were incubated overnight to allow for complete digestion of blood with Proteinase K. Additionally, at the end, extracted

DNA was eluted in two successive steps with 100 μL of molecular grade water. The Qubit® dsDNA Broad Range Assay Kit (Invitrogen) was used with the Qubit® 2.0 Fluorometer (Invitrogen) to determine the concentration of double stranded DNA (dsDNA) in each extracted sample.

Genome subsamples were prepared using double digest restriction-site associated DNA sequencing (ddRADSeq). We used the protocol introduced by Peterson et al. (2012) with two main differences. First, during the double digest phase, a minimum of 500 ng of extracted dsDNA from each sample was double digested with the restriction enzymes EcoRI and MspI for 3.5 hours at 37°C and then held at 4°C. Second, during adapter ligation, the combined digested DNA, adapters and unique barcodes, which would allow for individual sample identification during multiplexing, were incubated with T4 DNA ligase at 16°C for 16 hours. The T4 DNA ligase was then inactivated at 65°C for 10 minutes and then the temperature was decreased to 23°C at a rate of 1°C per minute to allow the dsDNA to reanneal.

After pooling, size selection was carried out with Pippin Prep (Sage Science) to retain a fragment peak of 420 bp (fragment range: 385 to 455 bp), which is within the optimal fragment size range for Illumina HiSeq 4000 sequencing. After another round of cleanup with magnetic beads, the size selected fragments were amplified with polymerase chain reaction (PCR) for 12 cycles. After PCR, the four concentration bands were pooled at equimolar volumes consisting of approximately 37 ng of DNA. Because one sample from the Northeast did not have sufficient dsDNA after restriction, only 47 individual libraries were successfully prepared (34 from the CCNR and 13 from the Northeast). The final multiplexed library was then sent to Novogene AIT Genomics Pte Ltd for sequencing on a single lane of Illumina HiSeq 4000 to produce 150 bp paired end reads. The library was spiked with 40% phiX to increase base diversity. The 47 unique barcodes (Table S1) and indexes (Table S2) can be found in the Appendix.

**Identification of single nucleotide polymorphisms.** The sequencing quality of the raw sequence reads was checked with FastQC (Babraham Bioinformatics) to determine Phred scores across all base positions. Using the process_radtags program in Stacks v1.44 (Catchen et al., 2013), sequenced reads were demultiplexed and cleaned by removing reads with an uncalled base or low-quality scores. To ensure a minimum of 99.9% accuracy in base calling, the sequenced reads were truncated to 145 bp, with interquartile Phred scores greater than 30. The demultiplexed sequences were then aligned to a pig (*Sus scrofa*) genome from Li et al. (2017) using the default settings in bwa.mem (Li & Durbin, 2009). To streamline the aligned sequences for further data processing, they were sorted according to coordinate order using samtools sort (Li et al., 2009).

To identify SNPs, refmap.pl with all three components (pstacks, cstacks and sstacks) in Stacks v1.44 (Catchen et al., 2013) was used. With the exception of -m (minimum stack depth), which is the minimum number of identical

aligned reads required to consider a stack of reads as a locus in the pstacks component, all other parameters were left at default. SNPs were identified at m=5 and m=10, with the latter being a stricter method of identifying loci. As sufficient loci were identified at m=10 (55,076 loci), we used this setting for downstream analyses. Loci were then processed using the populations program (Catchen et al., 2013) and retained only when a locus was present in at least 90% of individuals (-r). Individuals were partitioned into one population. The parameter to filter for a minimum minor allele frequency (-min_maf) was left at its default setting of 0.05. All other parameters were left at their default settings except the function write_single_snp, which was used to extract the first SNP from each locus, resulting in a set of unlinked SNPs.

The extracted SNPs were then filtered for linkage disequilibrium and checked for missing data in PLINK v1.9 (Chang et al., 2015). A total of five individuals (one from the CCNR and four from the Northeast) had more than 30% missing data and the analyses mentioned above were conducted again without these individuals, leaving 42 samples for downstream analyses. Pairwise genetic distances were also calculated using the identity-by-state (IBS) and Hamming distance calculation engine implemented in PLINK v1.9. BayeScan (Foll & Gaggiotti, 2008) was then used to check for selection neutrality in the SNP loci.

**Bioinformatic analysis.** To understand the population structure of individuals, a model-based clustering method was implemented in STRUCTURE v2.3.4 (Pritchard et al., 2000) where individuals were probabilistically assigned to a user-defined number of genetic clusters (K=1 to 5). Ten Markov Chain Monte-Carlo chains (replicates) were run for each value of K and each chain had a burn-in of 100,000 iterations followed by 500,000 more iterations for analysis. Additionally, the admixture model was used to jointly assign individuals that exhibited genetic admixture to two or more clusters. The marginal likelihood corresponding to the number of genetic clusters identified in STRUCTURE was calculated using the program Structure Harvester (Earl & vonHoldt, 2012). Observed ($H_o$) and expected ($H_e$) heterozygosities, were estimated for the CCNR and Northeast with the function bootstrapHet in the package PopGenKit (Paquette, 2012) in R 3.5.0 (R Core Team, 2018). Individuals were resampled with 1,000 bootstraps to estimate the mean $H_o$ and mean $H_e$ along with the 95% confidence intervals. To test for equal variances between the observed heterozygosities and expected heterozygosities of the two study sites, Bartlett's test of homogeneity of variances was first conducted with the function bartlett.test in the package adegenet (Jombart & Ahmed, 2011) in R 3.5.0. Because Bartlett's test found that the variances were significantly different, Welch's t-test (Welch, 1947), for unequal variance between samples, was conducted to test for significant differences between the observed heterozygosities and expected heterozygosities; this was performed using the function t.test in R 3.5.0.

To further investigate the genetic structure of individuals, a principal component analysis (PCA) of pairwise codominant genetic distances was conducted with the package SNPRelate (Zheng et al., 2012) in R 3.5.0. PCA coordinates were then plotted in R 3.5.0 with ggplot2 (Wickham, 2011). Finally, fixation indices ($F_{ST}$) were estimated using the R package hierfstat (Goudet, 2005) to investigate the genetic variation between the two sites. To calculate the statistical significance of the $F_{ST}$, the 95% confidence limits were calculated with 5,000 bootstraps using the function boot.ppfst found in the package hierfstat. Kinship values were also obtained using maximum likelihood estimation with the function snpgdsIBDSelection found in the package SNPRelate.

## RESULTS

Of 1,081 pairings, only 50 pairs had kinship values > 0.2 (14 individuals from the CCNR). However, the PCA excluding individuals with high kinship values did not differ from the PCA that included them (Fig. S1, Table S3). Therefore, the remaining analyses included individuals with high kinship values. The final PCA found two distinct genetic clusters (Fig. 2). The first genetic cluster (henceforth referred to as Cluster A) consisted of three individuals (P03, P06 and P07) from the Northeast, and all the individuals from the CCNR except P21 (Fig. 2). The second genetic cluster (henceforth referred to as Cluster B) consisted of the remaining individuals from the Northeast, excluding P09 and P13, which occurred with P21 in between the two genetic clusters (Fig. 2). On closer inspection of the individuals with kinship values < 0.2, P07 from the NE was closely related to individuals P18 (kinship value = 0.193), P27 (kinship value = 0.199) and P47 (kinship value = 0.189) from the CCNR (Table S3).

The population genomic analysis with STRUCTURE found that the marginal likelihood was maximised and the structure found in the data was best explained when there were two distinct genetic clusters (K=2) (Fig. S2). STRUCTURE identified the same genetic clusters as the PCA (Fig. 3). It depicted the individuals that occurred between the two clusters (P09, P13 and P21) from the PCA with a signature of genetic admixture between Clusters A and B. The genetic makeup of P09 (~67% Cluster B and ~33% Cluster A), P13 (~67% Cluster B and ~33% Cluster A) and P21 (~25% Cluster B and 75% Cluster A) in the STRUCTURE plot was in agreement with these samples' positions in the PCA plot (Fig. 2). The estimated $F_{ST}$ value between the Northeast and CCNR was 0.152 and was outside the 95% confidence limits (0.187 to 0.194). This indicates that the level of genetic differentiation between the two populations was significantly lower than expected and corroborates with the STRUCTURE and PCA analyses as five out of nine of the individuals in the Northeast were related to Cluster A.

Overall, the observed and expected heterozygosities of the CCNR population were lower than the Northeast population. Within each population, Welch's t-test found that the $H_o$ was significantly different from the $H_e$ (Northeast: $H_o$ = 0.245, $H_e$ = 0.308, p-value < $2.2 \times 10^{-16}$; CCNR: $H_o$ = 0.168, $H_e$ = 0.164, p-value = 0.042). Between populations, Welch's t-test found that the $H_o$ of the CCNR was significantly lower from
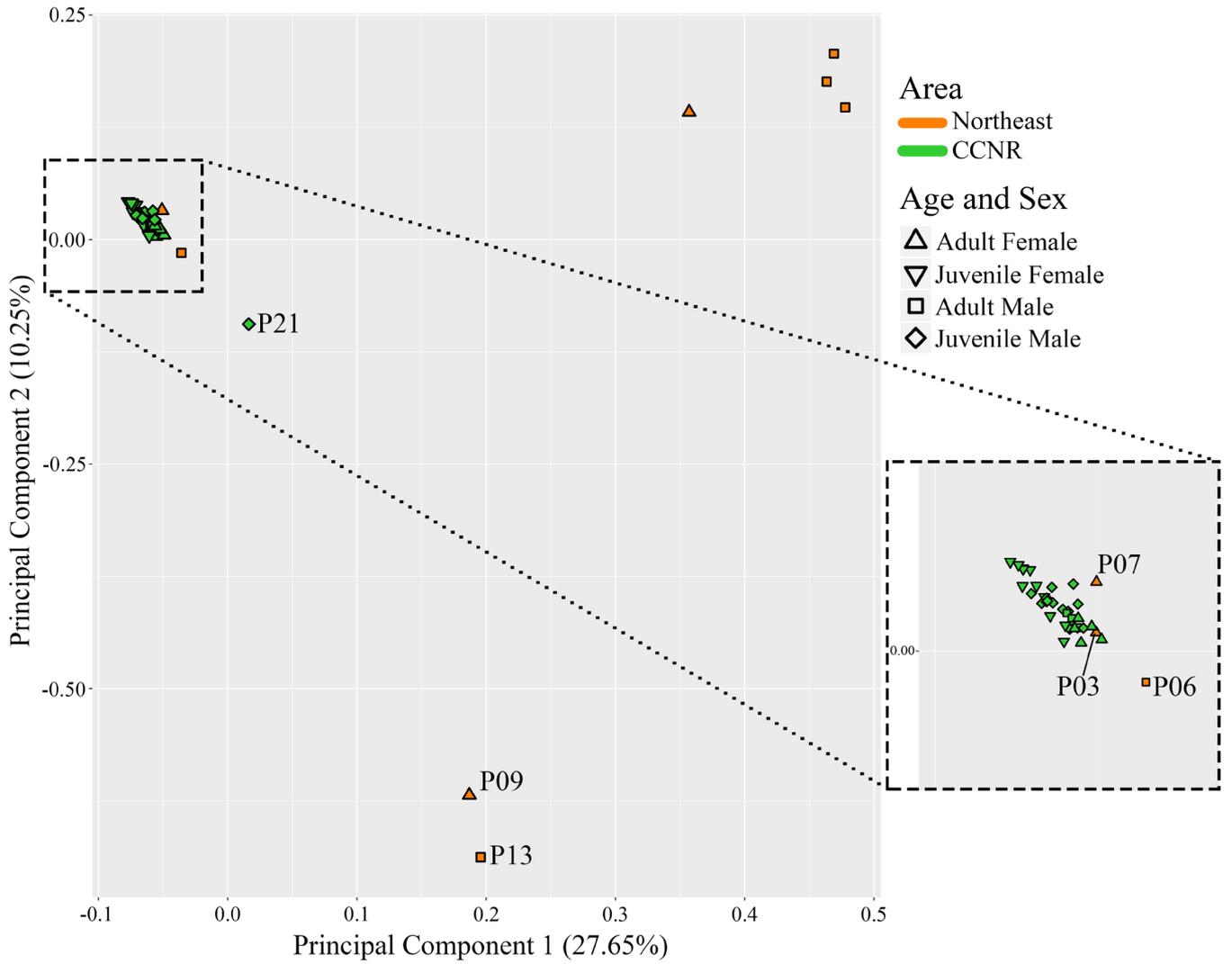
Fig. 2. Principal Component Analysis plot showing the 42 individuals from the Central Catchment Nature Reserve (CCNR) and the Northeast differentiated by sex and age class. Individuals exhibiting genetic admixture are labelled. Percentage variation accounted for by each principal component is indicated in brackets.
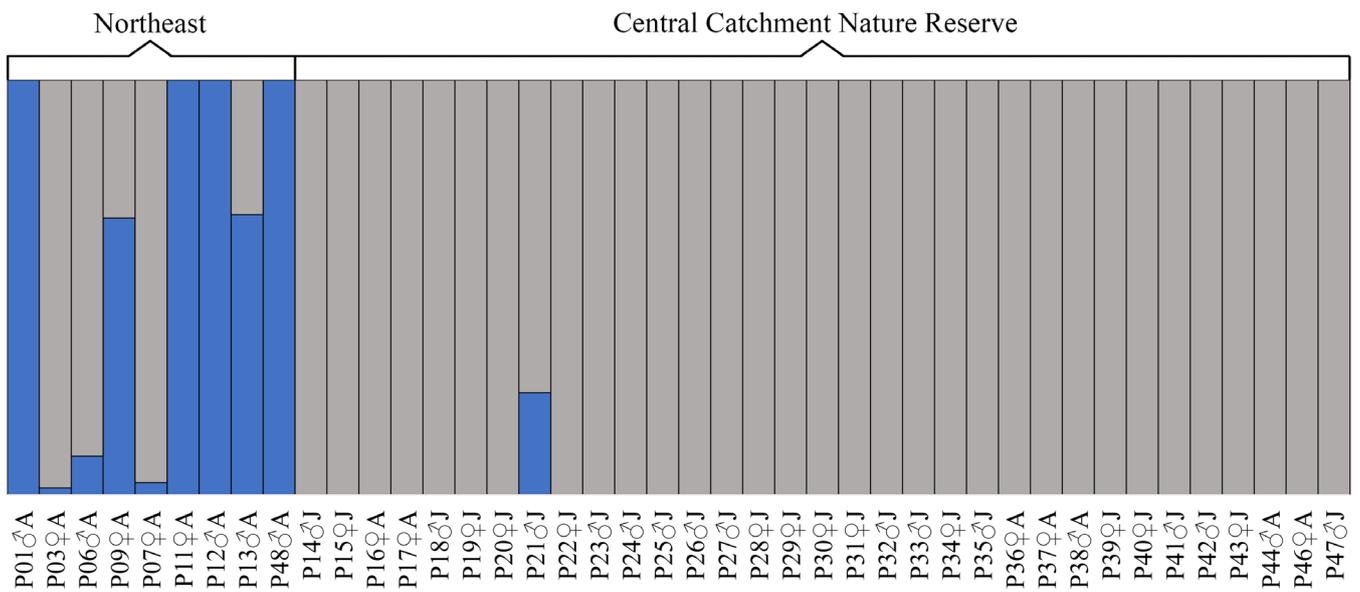


Fig. 3. STRUCTURE plot at K=2. Individuals are labelled according to 1) ID number, 2) sex and 3) age class (A for adult, J for juvenile). Sampling localities are indicated by brackets above the bars.
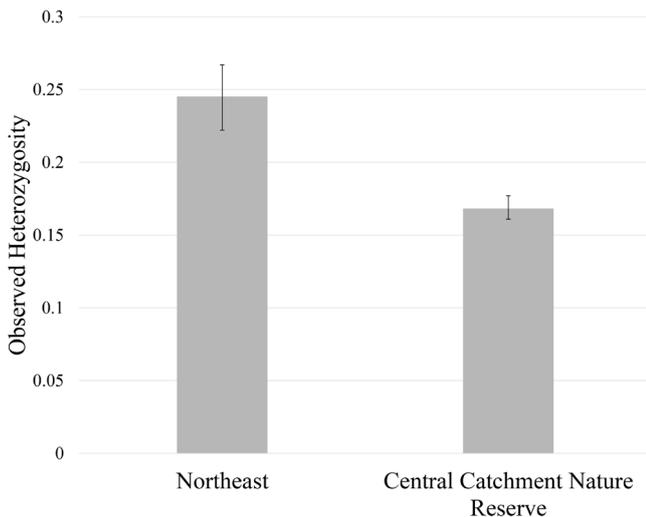
Fig. 4. Mean observed heterozygosities of the Northeast population and the Central Catchment Nature Reserve population. Error bars represent 95% confidence intervals of the mean.

the $H_o$ of the Northeast (t = -44.797, df = 53131, p-value < $2.2 \times 10^{-16}$) (Fig. 4).

## DISCUSSION

Our results indicated that while most samples from the CCNR had a homogenous genomic profile, samples from the Northeast showed a mixed signature that indicated partial affinity with the CCNR samples, and a significant genomic component unrelated to the CCNR. Given that pigs were previously locally extinct in the CCNR (Teo & Rajathurai, 1997), the similar genetic signatures in the CCNR and the Northeast imply that pigs from the Northeast dispersed across densely urbanised areas (approximately 9.68 km) and re-colonised the CCNR. Alternatively, both forest fragments may have been colonised by pigs from a similar source population. Both scenarios required pigs to have moved across the urban landscape. This is also supported by the kinship values of P07 from the NE to P18, P27, and P47 from the CCNR. P07 was an adult female while P18, P27 and P47 were juveniles. It is possible that they may have the same mother and/or father that dispersed from the NE to the CCNR. Alternatively, P07 may have mothered P18, P27 and P47 in the CCNR and then dispersed to the NE. It is important to note that juvenile pigs are weaned and can move independently of adults before reaching sub-adulthood and losing their stripes (Diong, 1973). This extends to the juvenile individual P21 (Fig. 3) which could have dispersed from the Northeast to the CCNR. Previous studies have shown that pigs can cross man-made infrastructure such as bridges, adapted culverts and highways (Mata et al., 2008; Corlatti et al., 2009; Frantz et al., 2012). Pigs have also been reported to exploit features such as riparian and forest corridors, hedges and ditches to travel over long distances of unfavorable habitat (Caley, 1997; Thurfjell et al., 2009; García et al., 2011). Therefore, the dispersal of pigs across Singapore's urban landscape may have been aided by man-made parks, waterways, and small forest patches in undeveloped land (Fig. 1). This hypothesis is also supported by evidence of a satellite collared-pig moving out of the CCNR into a nearby forest patch and then into a man-made park (Koh et al., 2018).

The pig population in the CCNR emerged as genetically less diverse than the pig population in the Northeast (Figs. 2, 3). Because this study examined populations of the same species, the differences in levels of genetic diversity between both populations can be caused by factors such as the initial population size, population bottlenecks and ecological disturbances (Furlan et al., 2012; Leffler et al., 2012; Romiguier et al., 2014). It is possible that the Northeast population receives migrants from surrounding islands and Malaysia while the CCNR population does not, resulting in the higher genetic diversity in the Northeast population. It is also possible, given the similar genetic signatures of the Northeast and CCNR populations, that the comparatively low genetic diversity of the CCNR population was caused by a small initial population that dispersed from the NE and other forest fragments. Despite the hypothesis from Harrison (1974), it is unlikely that the CCNR was repopulated by escapees from nearby pig farms. This is because all pig farming was phased out by 1990 (Taiganides, 1992) and no evidence of pigs were detected within the CCNR in the four-year survey conducted from 1993 to 1997 (Teo & Rajathurai, 1997). Because pigs have a high reproductive rate (Massei & Genov, 2004) and can heavily impact the environment (Crooks, 2002), it is also unlikely that their signs of rooting, wallowing and nest construction would have been missed in the four-year survey.

Without additional SNP data from pig populations in other forest fragments on Singapore's main island, Singapore's offshore islands and Malaysia, it is difficult to deduce the origins of both pig populations, and the direction and frequency of gene flow between the CCNR and Northeast populations.

**Management implications.** This study suggests that pigs are capable of dispersing between forest fragments (approximately 9.68 km apart) in a highly urbanised landscape. To aid in the understanding of the pigs' movements across Singapore, long term collaring studies should be conducted to identify the specific corridors used by the pigs for dispersal. These corridors can then be improved by planting additional shrubs and trees for cover, and/or constructing wildlife passes. The identification of specific corridors can also help mitigate road kill and vehicular impact accidents. Finally, a larger population genomic assessment should be conducted to understand pig dispersal within Singapore and how they re-colonised Singapore's main island.

This study has also shown that pigs are an exception to the general pattern shown by other large mammals, where there are usually significant impacts on dispersal and gene flow by urbanised areas (Huck et al., 2010; Ordeñana et al., 2010; Tammeleht et al., 2010). This result should be taken into consideration when implementing management strategies for pig populations.

## ACKNOWLEDGEMENTS

## LITERATURE CITED

Allendorf FW & Lundquist LL (2003) Introduction: Population biology, evolution, and control of invasive species. Conservation Biology, 17(1): 24–30.

Andrén H (1994) Effects of habitat fragmentation on birds and mammals in landscapes with different proportions of suitable habitat: a review. Oikos, 71(3): 355–366.

Arnold GW, Steven DE, Weeldenburg JR & Smith EA (1993) Influences of remnant size, spacing pattern and connectivity on population-boundaries and demography in euros *Macropus robustus* living in a fragmented landscape. Biological conservation, 64(3): 219–230.

Arrington DA, Toth LA & Koebel JW (1999) Effects of rooting by feral hogs *Sus scrofa* L. on the structure of a floodplain vegetation assemblage. Wetlands, 19(3): 535–544.

Barrios-Garcia MN & Ballari SA (2012) Impact of wild boar (*Sus scrofa*) in its introduced and native range: a review. Biological Invasions, 14(11): 2283–2300.

Brook BW, Sodhi NS & Ng PKL (2003) Catastrophic extinctions follow deforestation in Singapore. Nature, 424(6947): 420–426.

Brumfield RT, Beerli P, Nickerson DA & Edwards SV (2003) The utility of single nucleotide polymorphisms in inferences of population history. Trends in Ecology & Evolution, 18(5): 249–256.

Cahill S, Llimona F, Cabañeros L & Calomardo F (2012) Characteristics of wild boar (*Sus scrofa*) habituation to urban areas in the Collserola Natural Park (Barcelona) and comparison with other locations. Animal Biodiversity and Conservation, 35(2): 221–233.

Caley P (1997) Movements, activity patterns and habitat use of feral pigs (*Sus scrofa*) in a tropical habitat. Wildlife Research, 24: 77–87.

Catchen J, Hohenlohe PA, Bassham S, Amores A & Cresko WA (2013) Stacks: an analysis tool set for population genomics. Molecular Ecology, 22(11): 3124–3140.

Chang CC, Chow CC, Tellier LC, Vattikuti S, Purcell SM & Lee JJ (2015) Second-generation PLINK: rising to the challenge of larger and richer datasets. GigaScience, 4(1): 7.

Corlatti L, Hackländer K & Frey-Roos F (2009) Ability of wildlife overpasses to provide connectivity and prevent genetic isolation. Conservation Biology, 23(3): 548–556.

Corlett RT (1992) The ecological transformation of Singapore, 1819-1990. Journal of Biogeography, 19(4): 411–420.

Coulon A, Cosson JF, Angibault JM, Cargnelutti B, Galan M, Morellet N, Petit E, Aulagnier S & Hewison AJM (2004) Landscape connectivity influences gene flow in a roe deer population inhabiting a fragmented landscape: an individual–based approach. Molecular Ecology, 13(9): 2841–2850.

Crooks JA (2002) Characterizing ecosystem-level consequences of biological invasions: the role of ecosystem engineers. Oikos, 97(2): 153–166.

Diffendorfer JE, Gaines MS & Holt RD (1995) Habitat fragmentation and movements of three small mammals (*Sigmodon*, *Microtus*, and *Peromyscus*). Ecology, 76(3): 827–839.

Diong CH (1973) Studies of the Malayan wild pig in Perak and Johore. Malayan Nature Journal, 26: 120–151.

Earl DA & vonHoldt BM (2012) STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conservation Genetics Resources, 4(2): 359–361.

Epps CW, Palsbøll PJ, Wehausen JD, Roderick GK, Ramey RR & McCullough DR (2005) Highways block gene flow and cause a rapid decline in genetic diversity of desert bighorn sheep. Ecology Letters, 8(10): 1029–1038.

Foll M & Gaggiotti O (2008) A genome-scan method to identify selected loci appropriate for both dominant and codominant markers: a Bayesian perspective. Genetics, 180(2): 977–993.

Frantz AC, Bertouille S, Eloy MC, Licoppe A, Chaumont F & Flamand MC (2012) Comparative landscape genetic analyses show a Belgian motorway to be a gene flow barrier for red deer (*Cervus elaphus*), but not wild boars (*Sus scrofa*). Molecular Ecology, 21(14): 3445–3457.

Furlan E, Stoklosa J, Griffiths J, Gust N, Ellis R, Huggins RM & Weeks AR (2012) Small population size and extremely low levels of genetic diversity in island populations of the platypus, *Ornithorhynchus anatinus*. Ecology and Evolution, 2(4): 844–857.

García G, Vergara J & Lombardi R (2011) Genetic characterization and phylogeography of the wild boar *Sus scrofa* introduced into Uruguay. Genetics and Molecular Biology, 34(2): 329–337.

Gascon C & Lovejoy TE (1998) Ecological impacts of forest fragmentation in central Amazonia. Zoology, 101: 273–280.

Goudet J (2005) HIERFSTAT, a package for R to compute and test hierarchical F-statistics. Molecular Ecology, 5(1): 184–186.

Harrison J (1974) An Introduction to the Mammals of Singapore and Malaya. Malayan Nature Society, Singapore, 340 pp.

Huck M, Jędrzejewski W, Borowik T, Miłosz-Cielma M, Schmidt K, Jędrzejewska B, Nowak S & Mysłajek RW (2010) Habitat suitability, corridors and dispersal barriers for large carnivores in Poland. Acta Theriologica, 55(2): 177–192.

Jombart T & Ahmed I (2011) adegenet 1.3-1: new tools for the analysis of genome-wide SNP data. Bioinformatics, 27(21): 3070–3071.

Jules ES, Frost LS & Tallmon DA (1999) Ecological consequences of forest fragmentation in the Klamath region. Natural Areas Journal, 19: 368–378.

Keyghobadi N (2007) The genetic implications of habitat fragmentation for animals. Canadian Journal of Zoology, 85(10): 1049–1064.

Kjeldsen SR, Zenger KR, Leigh K, Ellis W, Tobey J, Phalen D, Melzer A, FitzGibbon S & Raadsma HW (2016) Genome-wide SNP loci reveal novel insights into koala (*Phascolarctos cinereus*) population variability across its range. Conservation Genetics, 17(2): 337–353.

Koh J-MJ, Webb EL & Leung K-PL (2018) Using a spatial mark-resight model to estimate the parameters of a wild pig (*Sus scrofa*) population in Singapore. Raffles Bulletin of Zoology, 66: 494–505.

Kotanen PM (1995) Responses of vegetation to a changing regime of disturbance: effects of feral pigs in a Californian coastal prairie. Ecography, 18(2): 190–199.

Kotulski Y & König A (2008) Conflicts, crises and challenges: wild boar in the Berlin City – a social empirical and statistical survey. Natura Croatica : Periodicum Musei Historiae Naturalis Croatici, 17(4): 233–246.

Kurki S, Nikula A, Helle P & Linden H (2000) Landscape fragmentation and forest composition effects on grouse breeding success in boreal forests. Ecology, 81: 1985–1997.

Leffler EM, Bullaughey K, Matute DR, Meyer WK, Ségurel L, Venkat A, Andolfatto P & Przeworski M (2012) Revisiting an old riddle: What determines genetic diversity levels within species? PLOS Biology, 10(9): e1001388.

Li H & Durbin R (2009) Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics, 25(14): 1754–1760.

Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, Marth G, Abecasis G & Durbin R (2009) The sequence alignment/map format and SAMtools. Bioinformatics, 25(16): 2078–2079.

Li M, Chen L, Tian S, Lin Y, Tang Q, Zhou X, Li D, Yeung CKL, Che T, Jin L, Fu Y, Ma J, Wang X, Jiang A, Lan J, Pan Q, Liu Y, Luo Z, Guo Z, Liu H, Zhu L, Shuai S, Tang G, Zhao J, Jiang Y, Bai L, Zhang S, Mai M, Li C, Wang D, Gu Y, Wang G, Lu H, Li Y, Zhu H, Li Z, Li M, Gladyshev VN, Jiang Z, Zhao S, Wang J, Li R & Li X (2017) Comprehensive variation discovery and recovery of missing sequence in the pig genome using multiple de novo assemblies. Genome Research, 27(5): 865–874.

Loker CA & Decker DJ (1998) Changes in human activity and the 'not–in–my–backyard' wildlife syndrome: suburban residents' perspectives on wildlife. Gibier Faune Sauvage, 15: 725–734.

Mata C, Hervás I, Herranz J, Suárez F & Malo JE (2008) Are motorway wildlife passages worth building? Vertebrate use of road-crossing structures on a Spanish motorway. Journal of Environmental Management, 88(3): 407–415.

Massei G & Genov PV (2004) The environmental impact of wild boar. Galemys, 16: 135–145.

Matías L, Zamora R, Mendoza I & Hódar JA (2010) Seed dispersal patterns by large frugivorous mammals in a degraded mosaic landscape. Restoration Ecology, 18(5): 619–627.

Morin PA, Luikart G, Wayne RK & the SNPwg (2004) SNPs in ecology, evolution and conservation. Trends in Ecology & Evolution, 19(4): 208–216.

Neaves LE, Zenger KR, Prince RIT, Eldridge MDB & Cooper DW (2009) Landscape discontinuities influence gene flow and genetic structure in a large, vagile Australian mammal, *Macropus fuliginosus*. Molecular Ecology, 18(16): 3363–3378.

Ng EYX, Garg KM, Low GW, Chattopadhyay B, Oh RRY, Lee JGH & Rheindt FE (2017) Conservation genomics identifies impact of trade in a threatened songbird. Biological conservation, 214(Supplement C): 101–108.

Ordeñana MA, Crooks KR, Boydston EE, Fisher RN, Lyren LM, Siudyla S, Haas CD, Harris S, Hathaway SA, Turschak GM, Miles AK & Van Vuren DH (2010) Effects of urbanisation on carnivore species distribution and richness. Journal of Mammalogy, 91(6): 1322–1331.

Paetkau D, Calvert W, Stirling I & Strobeck C (1995) Microsatellite analysis of population structure in Canadian polar bears. Molecular Ecology, 4(3): 347–354.

Paquette SR (2012) PopGenKit: Useful functions for (batch) file conversion and data resampling in microsatellite datasets. CRAN. https://rdrr.io/cran/PopGenKit/. (Accessed 4 June 2018).

Pérez-espona S, Pérez-barbería FJ, Mcleod JE, Jiggins CD, Gordon IJ & Pemberton JM (2008) Landscape features affect gene flow of Scottish Highland red deer (*Cervus elaphus*). Molecular Ecology, 17(4): 981–996.

Peterson BK, Weber JN, Kay EH, Fisher HS & Hoekstra HE (2012) Double digest RADseq: an inexpensive method for de novo SNP discovery and genotyping in model and non-model species. PLoS One, 7(5): e37135.

Pritchard JK, Stephens M & Donnelly P (2000) Inference of population structure using multilocus genotype data. Genetics, 155(2): 945–959.

R Core Team (2018) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.r-project.org/. (Accessed 6 May 2018).

Radeloff VC, Hammer RB, Stewart SI, Fried JS, Holcomb SS & McKeefry JF (2005) The wildland-urban interface in the United States. Ecological Applications, 15(3): 799–805.

Romiguier J, Gayral P, Ballenghien M, Bernard A, Cahais V, Chenuil A, Chiari Y, Dernat R, Duret L, Faivre N, Loire E, Lourenco JM, Nabholz B, Roux C, Tsagkogeorga G, Weber AA-T, Weinert LA, Belkhir K, Bierne N, Glémin S & Galtier N (2014) Comparative population genomics in animals uncovers the determinants of genetic diversity. Nature, 515(7526): 261–263.

Singapore Department of Statistics (2018) Latest Data. In: Statistics. http://www.singstat.gov.sg/statistics/latest-data#16. (Accessed 1 November 2018).

Slatkin M (1987) Gene flow and the geographic structure of natural populations. Science, 236(4803): 787–792.

Taiganides EP (1992) Pig waste management and recycling: the Singapore experience. International Development Research Centre, Ottawa, Canada, xiv + 368 pp.

Tammeleht E, Remm J, Korsten M, Davison J, Tumanov I, Saveljev A, Männil, Kojola I & Saarma U (2010) Genetic structure in large, continuous mammal populations: the example of brown bears in northwestern Eurasia. Molecular Ecology, 19(24): 5359–5370.

Teo RCH & Rajathurai S (1997) Mammals, reptiles and amphibians in the Nature Reserves of Singapore - Diversity, abundance and distribution. Gardens' Bulletin Singapore, 49: 353–425.

Thurfjell H, Ball J, Åhlén P-A, Kornacher P, Dettki H & Sjöberg K (2009) Habitat use and spatial patterns of wild boar *Sus scrofa* (L.): agricultural fields and edges. European Journal of Wildlife Research, 55(5): 517–523.

Turner IM, Wong YK, Chew PT & Ibrahim A (1997) Tree species richness in primary and old secondary tropical forest in Singapore. Biodiversity and Conservation, 6: 537–543.

Wade TG, Ritters KH, Wickham JD & Jones KB (2003) Distribution and causes of global forest fragmentation. Conservation Ecology, 7(2): 7.

Wagner CE, Keller I, Wittwer S, Selz OM, Mwaiko S, Greuter L, Sivasundar A & Seehausen O (2013) Genome-wide RAD sequence data provide unprecedented resolution of species boundaries and relationships in the Lake Victoria cichlid adaptive radiation. Molecular Ecology, 22(3): 787–798.

Waits L, Taberlet P, Swenson JE, Sandegren F & Franzén R (2000) Nuclear DNA microsatellite analysis of genetic diversity and gene flow in the Scandinavian brown bear (*Ursus arctos*). Molecular Ecology, 9(4): 421–431.

Wallace AR (1895) Island Life: Or the Phenomena and Causes of Insular Faunas and Floras Including a Revision and Attempted Solution of the Problem of Geological Climates. 2nd Edition. MacMillan and Co., London and New York, 73 pp.

Welander J (1995) Are wild boars a future threat to the Swedish flora? IBEX Journal of Mountain Ecology, 3: 165–167.

Welch BL (1947) The generalization of 'student's' problem when several different population variances are involved. Biometrika, 34(1-2): 28–35.

Wickham H (2011) ggplot2. Wiley Interdisciplinary Reviews: Computational Statistics, 3(2): 180–185.

Yee ATK, Corlett RT, Liew SC & Tan HTW (2011) The vegetation of Singapore - an updated map. Gardens' Bulletin Singapore, 63(1 & 2): 205–212.

Yong DL, Lee BPY-H, Ang A & Tan KH (2010) The status on Singapore island of the Eurasian wild pig *Sus scrofa* (Mammalia: Suidae). Nature in Singapore, 3: 227–237.

Zheng X, Levine D, Shen J, Gogarten SM, Laurie C & Weir BS (2012) A high-performance computing toolset for relatedness and principal component analysis of SNP data. Bioinformatics, 28(24): 3326–3328.

**APPENDIX**

Table S1. The 47 unique barcodes used in the library preparation.

| Barcode Name | Oligo Sequence |
| --- | --- |
| GCATG_EcoRI_P1.1 | ACACTCTTTCCCTACACGACGCTCTTCCGATCTGCATG |
| AACCA_EcoRI_P1.1 | ACACTCTTTCCCTACACGACGCTCTTCCGATCTAACCA |
| CGATC_EcoRI_P1.1 | ACACTCTTTCCCTACACGACGCTCTTCCGATCTCGATC |
| TCGAT_EcoRI_P1.1 | ACACTCTTTCCCTACACGACGCTCTTCCGATCTTCGAT |
| TGCAT_EcoRI_P1.1 | ACACTCTTTCCCTACACGACGCTCTTCCGATCTTGCAT |
| CAACC_EcoRI_P1.1 | ACACTCTTTCCCTACACGACGCTCTTCCGATCTCAACC |
| GGTTG_EcoRI_P1.1 | ACACTCTTTCCCTACACGACGCTCTTCCGATCTGGTTG |
| AAGGA_EcoRI_P1.1 | ACACTCTTTCCCTACACGACGCTCTTCCGATCTAAGGA |
| AGCTA_EcoRI_P1.1 | ACACTCTTTCCCTACACGACGCTCTTCCGATCTAGCTA |
| ACACA_EcoRI_P1.1 | ACACTCTTTCCCTACACGACGCTCTTCCGATCTACACA |
| AATTA_EcoRI_P1.1 | ACACTCTTTCCCTACACGACGCTCTTCCGATCTAATTA |
| ACGGT_EcoRI_P1.1 | ACACTCTTTCCCTACACGACGCTCTTCCGATCTACGGT |
| ACTGG_EcoRI_P1.1 | ACACTCTTTCCCTACACGACGCTCTTCCGATCTACTGG |
| ACTTC_EcoRI_P1.1 | ACACTCTTTCCCTACACGACGCTCTTCCGATCTACTTC |
| ATACG_EcoRI_P1.1 | ACACTCTTTCCCTACACGACGCTCTTCCGATCTATACG |
| ATGAG_EcoRI_P1.1 | ACACTCTTTCCCTACACGACGCTCTTCCGATCTATGAG |
| ATTAC_EcoRI_P1.1 | ACACTCTTTCCCTACACGACGCTCTTCCGATCTATTAC |
| CATAT_EcoRI_P1.1 | ACACTCTTTCCCTACACGACGCTCTTCCGATCTCATAT |
| CGAAT_EcoRI_P1.1 | ACACTCTTTCCCTACACGACGCTCTTCCGATCTCGAAT |
| CGGCT_EcoRI_P1.1 | ACACTCTTTCCCTACACGACGCTCTTCCGATCTCGGCT |
| CGGTA_EcoRI_P1.1 | ACACTCTTTCCCTACACGACGCTCTTCCGATCTCGGTA |
| CGTAC_EcoRI_P1.1 | ACACTCTTTCCCTACACGACGCTCTTCCGATCTCGTAC |
| CGTCG_EcoRI_P1.1 | ACACTCTTTCCCTACACGACGCTCTTCCGATCTCGTCG |
| CTGAT_EcoRI_P1.1 | ACACTCTTTCCCTACACGACGCTCTTCCGATCTCTGAT |
| CTGCG_EcoRI_P1.1 | ACACTCTTTCCCTACACGACGCTCTTCCGATCTCTGCG |
| CTGTC_EcoRI_P1.1 | ACACTCTTTCCCTACACGACGCTCTTCCGATCTCTGTC |
| CTTGG_EcoRI_P1.1 | ACACTCTTTCCCTACACGACGCTCTTCCGATCTCTTGG |
| GACAC_EcoRI_P1.1 | ACACTCTTTCCCTACACGACGCTCTTCCGATCTGACAC |
| GAGAT_EcoRI_P1.1 | ACACTCTTTCCCTACACGACGCTCTTCCGATCTGAGAT |
| GAGTC_EcoRI_P1.1 | ACACTCTTTCCCTACACGACGCTCTTCCGATCTGAGTC |
| GCCGT_EcoRI_P1.1 | ACACTCTTTCCCTACACGACGCTCTTCCGATCTGCCGT |
| GCTGA_EcoRI_P1.1 | ACACTCTTTCCCTACACGACGCTCTTCCGATCTGCTGA |
| GGATA_EcoRI_P1.1 | ACACTCTTTCCCTACACGACGCTCTTCCGATCTGGATA |
| GGCCA_EcoRI_P1.1 | ACACTCTTTCCCTACACGACGCTCTTCCGATCTGGCCA |
| GGCTC_EcoRI_P1.1 | ACACTCTTTCCCTACACGACGCTCTTCCGATCTGGCTC |
| GTAGT_EcoRI_P1.1 | ACACTCTTTCCCTACACGACGCTCTTCCGATCTGTAGT |
| GTCCG_EcoRI_P1.1 | ACACTCTTTCCCTACACGACGCTCTTCCGATCTGTCCG |

| Barcode Name | Oligo Sequence |
|---|---|
| GTCGA_EcoRI_P1.1 | ACACTCTTTCCCTACACGACGCTCTTCCGATCTGTCGA |
| TACCG_EcoRI_P1.1 | ACACTCTTTCCCTACACGACGCTCTTCCGATCTTACCG |
| TACGT_EcoRI_P1.1 | ACACTCTTTCCCTACACGACGCTCTTCCGATCTTACGT |
| TAGTA_EcoRI_P1.1 | ACACTCTTTCCCTACACGACGCTCTTCCGATCTTAGTA |
| TATAC_EcoRI_P1.1 | ACACTCTTTCCCTACACGACGCTCTTCCGATCTTATAC |
| TCACG_EcoRI_P1.1 | ACACTCTTTCCCTACACGACGCTCTTCCGATCTTCACG |
| TCAGT_EcoRI_P1.1 | ACACTCTTTCCCTACACGACGCTCTTCCGATCTTCAGT |
| TCCGG_EcoRI_P1.1 | ACACTCTTTCCCTACACGACGCTCTTCCGATCTTCCGG |
| TCTGC_EcoRI_P1.1 | ACACTCTTTCCCTACACGACGCTCTTCCGATCTTCTGC |

Table S2. The indexes used for both polymerase chain reactions in the library preparation.

| Index Name | Oligo Sequence |
|---|---|
| PCR 1 | AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACG |
| PCR2_Idx_ATCACG | CAAGCAGAAGACGGCATACGAGATCGTGATGTGACTGGAGTTCAGACGTGTGC |
| PCR2_Idx_2_CGATGT | CAAGCAGAAGACGGCATACGAGATACATCGGTGACTGGAGTTCAGACGTGTGC |
| PCR2_Idx_3_TTAGGC | CAAGCAGAAGACGGCATACGAGATGCCTAAGTGACTGGAGTTCAGACGTGTGC |
| PCR2_Idx_4_TGACCA | CAAGCAGAAGACGGCATACGAGATTGGTCAGTGACTGGAGTTCAGACGTGTGC |
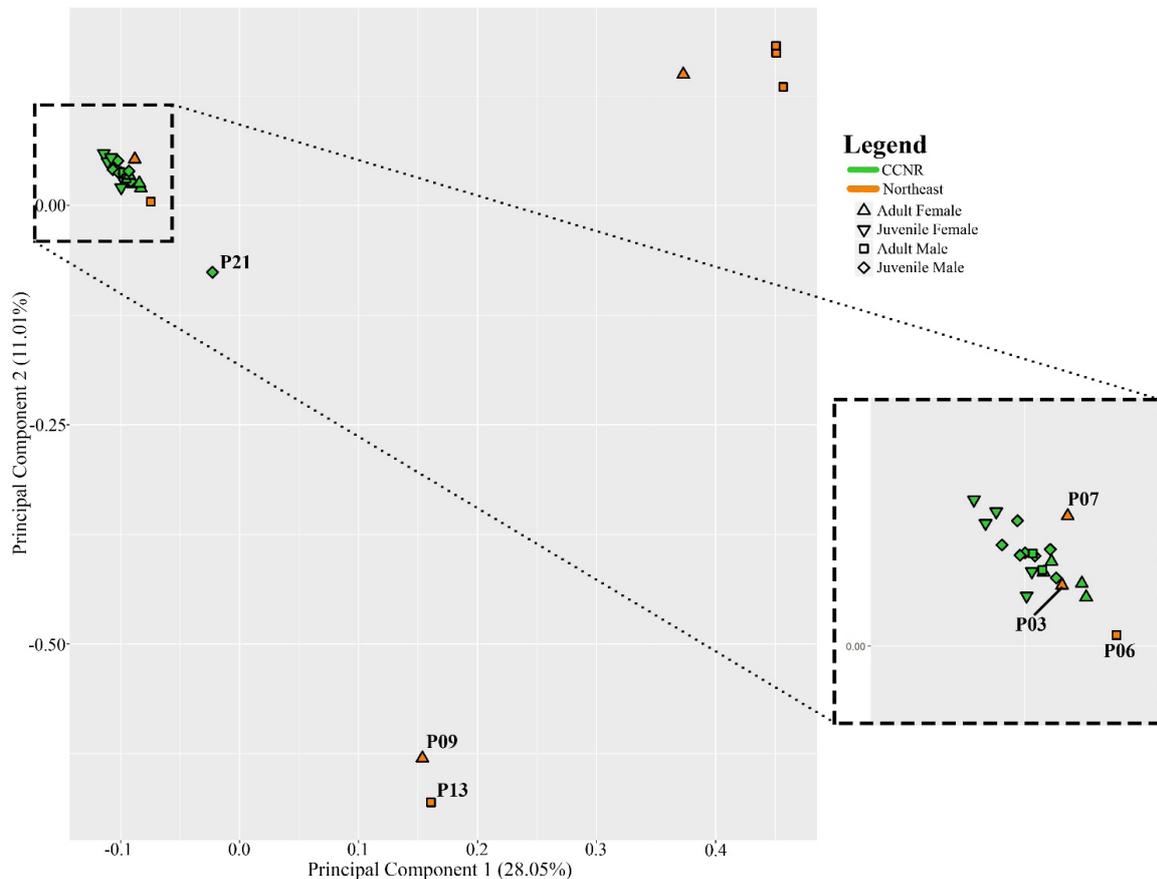


Fig. S1. Principal Component Analysis plot showing 28 out of 42 individuals from the Central Catchment Nature Reserve (CCNR) and the Northeast with kinship values < 0.2. Individuals are differentiated by sex and age class. Individuals exhibiting genetic admixture are labelled. Percentage variation accounted for by each principal component is indicated in brackets.
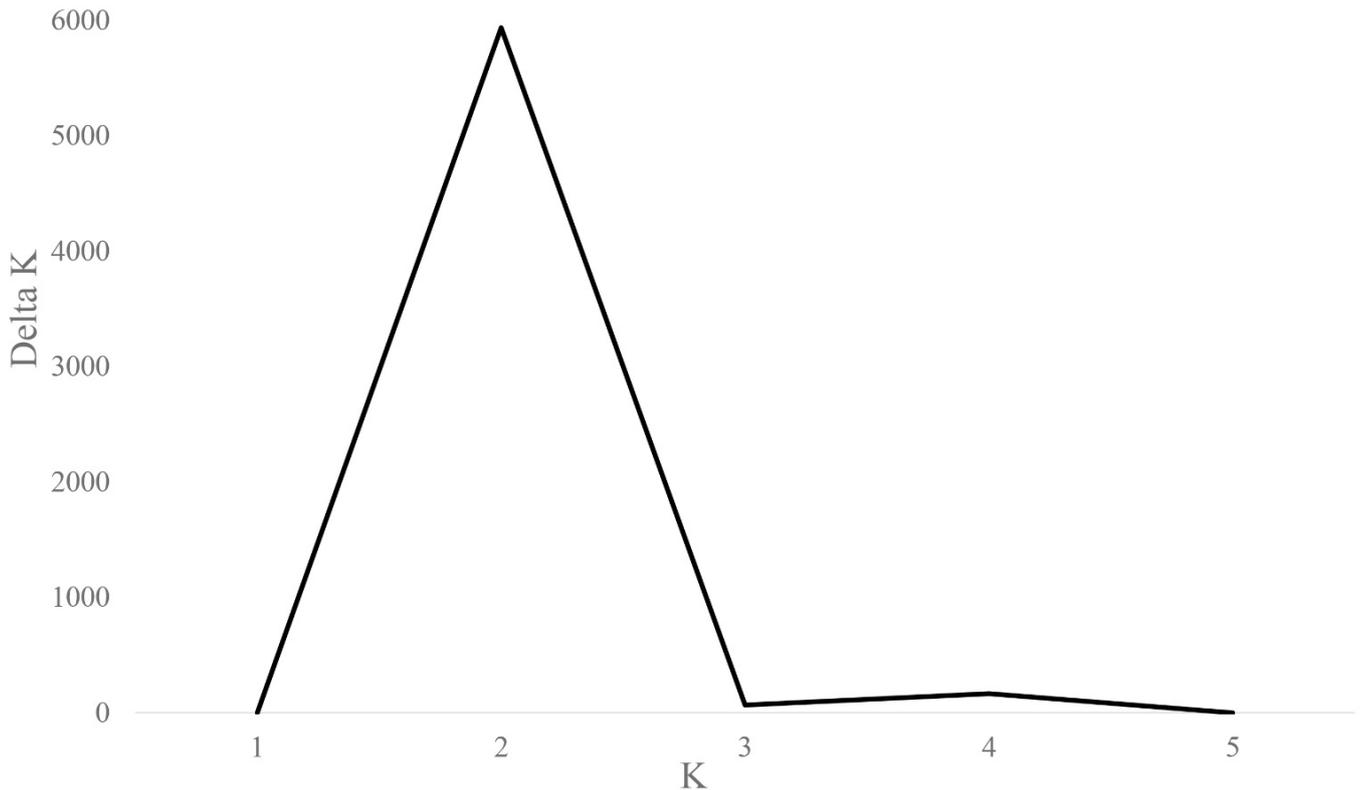
Fig. S2. Plot of Delta K from STRUCTURE output showing the Delta K values for the corresponding K values. The higher the Delta K value, the better the genetic structure is explained.

Inbreeding coefficients were also calculated for each individual and averaged for each site using the program Coancestry 1.0.1.8 (Wang, 2011). Using the allelic frequencies from the empirical data, a simulated dataset with known relatedness estimates was generated and analysed to determine the estimator that best fit the empirical data. From the analysis of the simulated dataset, the dyadic likelihood estimator (Milligan, 2003) was the most accurate and subsequently used to estimate inbreeding coefficients.
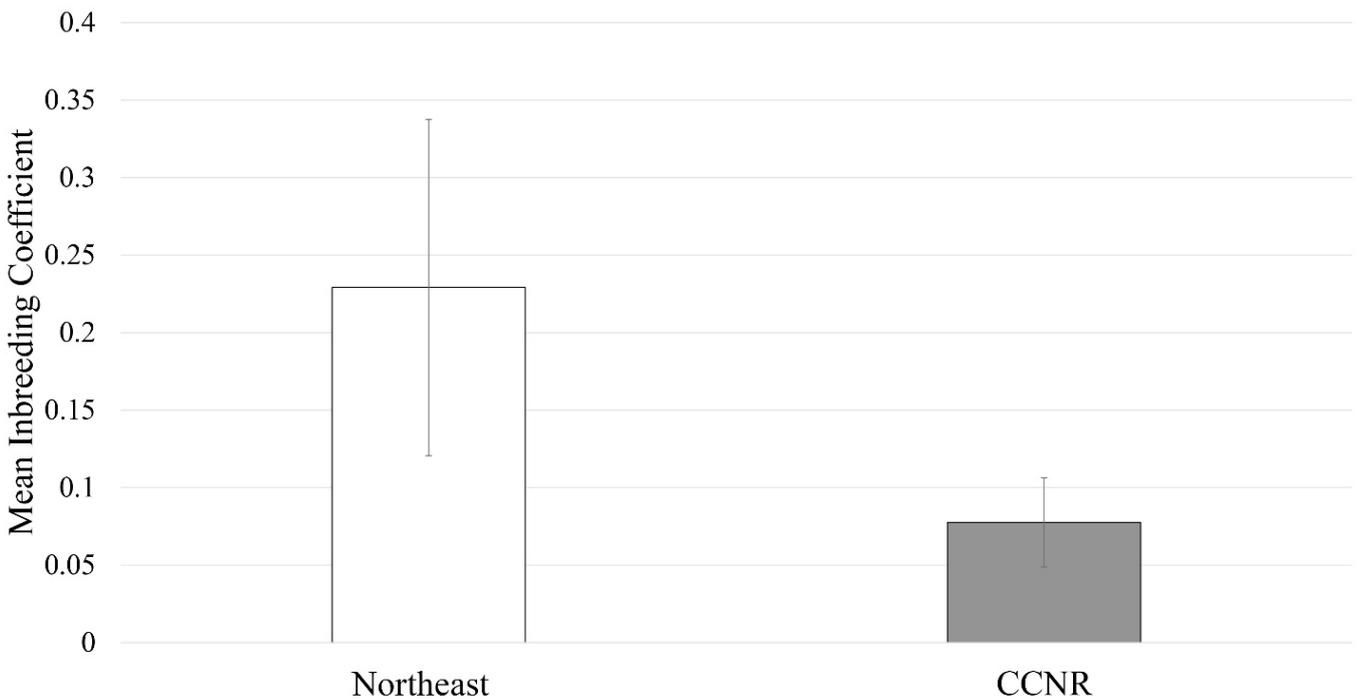


Fig. S3. Mean inbreeding coefficients of the populations of pigs found in the Northeast and the CCNR (Central Catchment Nature Reserve). Error bars represent 95% confidence interval.
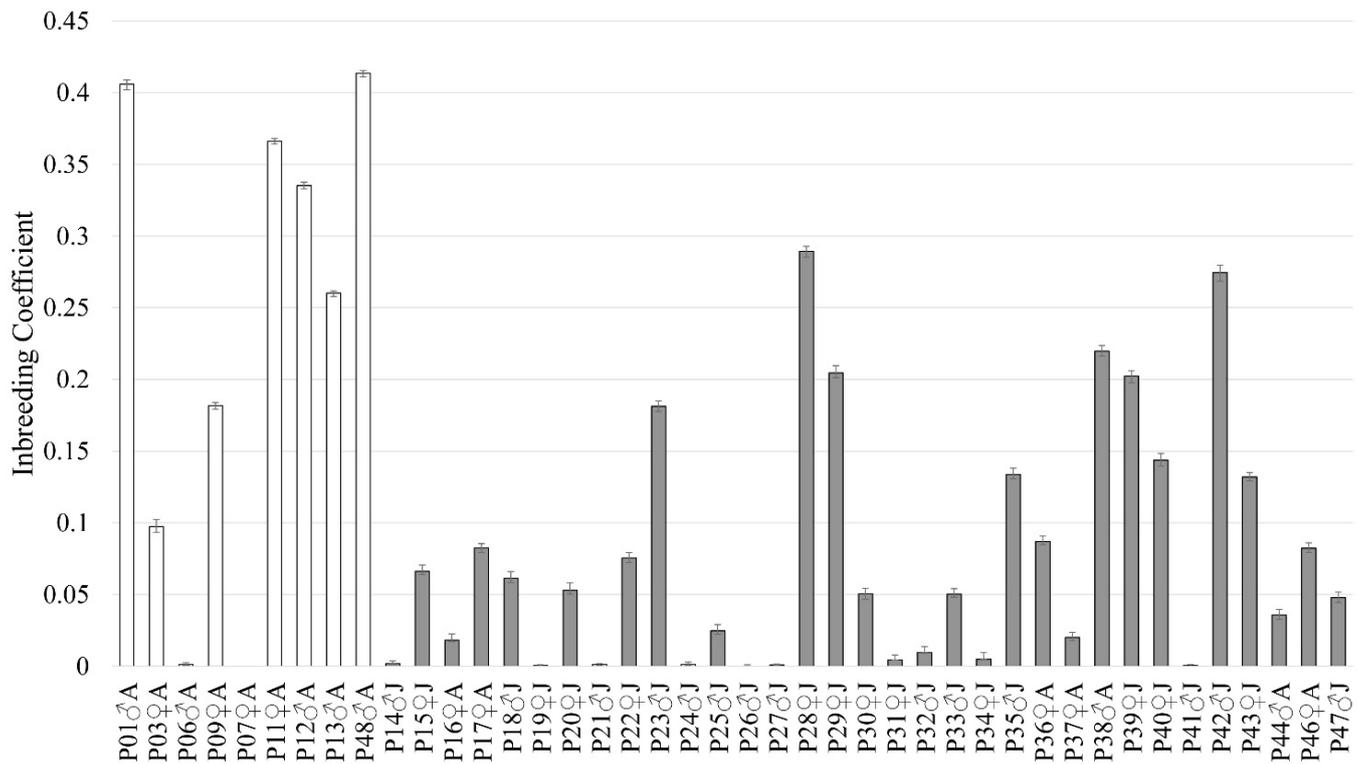
Fig. S4. Inbreeding coefficients of each individual. White bars represent individuals from the Northeast and grey bars represent individuals from the Central Catchment Nature Reserve. Error bars represent 95% confidence interval.

The inbreeding coefficients were not used in the main text as they were skewed and inconsistent with the results from STRUCTURE and the principal component analysis. The main reason why the inbreeding coefficients were biased is due to sample size (Northeast had nine individuals and CCNR had 33 individuals); where small sample sizes can result in biased genetic estimators (Da Costa et al., 2015).

**References**

Da Costa LS, Corneleo NS & Stefenon VM (2015) Conservation of Forest Biodiversity: how sample size affects the estimation of genetic parameters. Anais da Academia Brasileira de Ciências, 87: 1095–1100.

Milligan BG (2003) Maximum-likelihood estimation of relatedness. Genetics, 163: 1153–1167.

Wang J (2011) coancestry: a program for simulating, estimating and analysing relatedness and inbreeding coefficients. Molecular Ecology Resources, 11: 141–145. doi:10.1111/j.17550998.2010.02885.x.

Table S3. Individuals included in the Principal Component Analysis of Fig. S1, their pairing with other individuals and the corresponding kinship values.

| Individual 1 | Individual 2 | Kinship Value | Individual 1 | Individual 2 | Kinship Value |
|---|---|---|---|---|---|
| P07Lh | P27Pi | 0.19979 | P26Pi | P35Pi | 0.13755 |
| P28Pi | P39Pi | 0.199058 | P16Pi | P27Pi | 0.136666 |
| P11Lh | P12Lh | 0.19833 | P01Pu | P48Ci | 0.134703 |
| P28Pi | P31Pi | 0.198295 | P07Lh | P24Pi | 0.133451 |
| P18Pi | P47Pi | 0.197958 | P07Lh | P28Pi | 0.131268 |
| P18Pi | P27Pi | 0.197759 | P22Pi | P44Pi | 0.129368 |
| P31Pi | P39Pi | 0.196587 | P28Pi | P47Pi | 0.128922 |
| P27Pi | P47Pi | 0.196552 | P35Pi | P43Pi | 0.12864 |
| P37Pi | P46Pi | 0.196189 | P24Pi | P44Pi | 0.123863 |
| P22Pi | P46Pi | 0.195421 | P03Pu | P38Pi | 0.122892 |
| P07Lh | P18Pi | 0.193219 | P26Pi | P38Pi | 0.122874 |
| P11Lh | P48Ci | 0.190306 | P24Pi | P26Pi | 0.122823 |
| P07Lh | P47Pi | 0.188531 | P36Pi | P43Pi | 0.121752 |
| P26Pi | P28Pi | 0.185137 | P36Pi | P44Pi | 0.118587 |
| P27Pi | P39Pi | 0.177524 | P07Lh | P16Pi | 0.118196 |
| P24Pi | P28Pi | 0.176009 | P22Pi | P37Pi | 0.118126 |
| P24Pi | P31Pi | 0.171916 | P31Pi | P37Pi | 0.11779 |
| P22Pi | P25Pi | 0.170786 | P37Pi | P44Pi | 0.117103 |
| P01Pu | P12Lh | 0.170332 | P35Pi | P38Pi | 0.115545 |
| P27Pi | P31Pi | 0.169812 | P24Pi | P47Pi | 0.114334 |
| P16Pi | P46Pi | 0.166511 | P25Pi | P26Pi | 0.113055 |
| P31Pi | P47Pi | 0.16487 | P22Pi | P43Pi | 0.112969 |
| P26Pi | P44Pi | 0.161385 | P43Pi | P44Pi | 0.111949 |
| P26Pi | P39Pi | 0.161301 | P24Pi | P37Pi | 0.109409 |
| P07Lh | P39Pi | 0.159502 | P26Pi | P43Pi | 0.109395 |
| P01Pu | P11Lh | 0.159395 | P24Pi | P39Pi | 0.10905 |
| P24Pi | P25Pi | 0.153777 | P38Pi | P44Pi | 0.108731 |
| P22Pi | P24Pi | 0.153706 | P25Pi | P38Pi | 0.108269 |
| P36Pi | P37Pi | 0.153149 | P24Pi | P46Pi | 0.107991 |
| P12Lh | P48Ci | 0.152094 | P22Pi | P36Pi | 0.107848 |
| P07Lh | P31Pi | 0.152092 | P18Pi | P24Pi | 0.105931 |
| P24Pi | P27Pi | 0.150239 | P35Pi | P44Pi | 0.105197 |
| P27Pi | P28Pi | 0.145479 | P16Pi | P31Pi | 0.103871 |
| P16Pi | P47Pi | 0.145075 | P44Pi | P46Pi | 0.100323 |
| P36Pi | P46Pi | 0.142515 | P31Pi | P35Pi | 0.098549 |
| P39Pi | P47Pi | 0.141675 | P25Pi | P44Pi | 0.09666 |
| P09Pu | P13Lh | 0.140059 | P16Pi | P25Pi | 0.096482 |

| Individual 1 | Individual 2 | Kinship Value | Individual 1 | Individual 2 | Kinship Value |
|---|---|---|---|---|---|
| P25Pi | P43Pi | 0.095636 | P16Pi | P39Pi | 0.07201 |
| P18Pi | P37Pi | 0.094463 | P22Pi | P35Pi | 0.071821 |
| P26Pi | P31Pi | 0.093212 | P03Pu | P07Lh | 0.071548 |
| P25Pi | P31Pi | 0.092763 | P25Pi | P36Pi | 0.068903 |
| P16Pi | P24Pi | 0.092153 | P03Pu | P37Pi | 0.067682 |
| P16Pi | P22Pi | 0.091946 | P18Pi | P28Pi | 0.06767 |
| P35Pi | P36Pi | 0.091139 | P37Pi | P43Pi | 0.067585 |
| P31Pi | P43Pi | 0.091139 | P24Pi | P43Pi | 0.066621 |
| P06Pu | P26Pi | 0.090519 | P25Pi | P46Pi | 0.066108 |
| P06Pu | P43Pi | 0.089638 | P22Pi | P26Pi | 0.065955 |
| P22Pi | P31Pi | 0.089219 | P06Pu | P31Pi | 0.065941 |
| P18Pi | P44Pi | 0.088916 | P18Pi | P38Pi | 0.065759 |
| P28Pi | P35Pi | 0.087743 | P25Pi | P35Pi | 0.065744 |
| P18Pi | P39Pi | 0.087697 | P44Pi | P47Pi | 0.065559 |
| P16Pi | P38Pi | 0.085385 | P25Pi | P37Pi | 0.064491 |
| P16Pi | P18Pi | 0.085239 | P03Pu | P25Pi | 0.063489 |
| P03Pu | P26Pi | 0.084611 | P31Pi | P46Pi | 0.0625 |
| P03Pu | P27Pi | 0.08441 | P35Pi | P39Pi | 0.062146 |
| P38Pi | P43Pi | 0.08438 | P03Pu | P31Pi | 0.060948 |
| P03Pu | P18Pi | 0.084355 | P18Pi | P36Pi | 0.060312 |
| P28Pi | P38Pi | 0.082752 | P06Pu | P24Pi | 0.060236 |
| P24Pi | P35Pi | 0.082607 | P28Pi | P43Pi | 0.059558 |
| P16Pi | P43Pi | 0.081887 | P07Lh | P26Pi | 0.058593 |
| P18Pi | P43Pi | 0.081509 | P03Pu | P44Pi | 0.058319 |
| P38Pi | P39Pi | 0.079806 | P22Pi | P28Pi | 0.058098 |
| P39Pi | P43Pi | 0.079743 | P26Pi | P47Pi | 0.057669 |
| P18Pi | P35Pi | 0.079685 | P43Pi | P46Pi | 0.057642 |
| P36Pi | P38Pi | 0.079339 | P18Pi | P26Pi | 0.057327 |
| P06Pu | P35Pi | 0.07926 | P35Pi | P47Pi | 0.057237 |
| P03Pu | P06Pu | 0.07752 | P22Pi | P47Pi | 0.056759 |
| P18Pi | P31Pi | 0.077255 | P27Pi | P35Pi | 0.056457 |
| P16Pi | P28Pi | 0.076806 | P25Pi | P47Pi | 0.055478 |
| P03Pu | P39Pi | 0.076311 | P25Pi | P28Pi | 0.055365 |
| P06Pu | P38Pi | 0.075951 | P03Pu | P43Pi | 0.053428 |
| P16Pi | P37Pi | 0.075278 | P37Pi | P39Pi | 0.052888 |
| P26Pi | P27Pi | 0.074322 | P27Pi | P38Pi | 0.052631 |
| P03Pu | P28Pi | 0.074299 | P37Pi | P47Pi | 0.052336 |
| P03Pu | P16Pi | 0.072924 | P03Pu | P22Pi | 0.052309 |
| P22Pi | P38Pi | 0.072035 | P06Pu | P18Pi | 0.051949 |

| Individual 1 | Individual 2 | Kinship Value | Individual 1 | Individual 2 | Kinship Value |
|---|---|---|---|---|---|
| P03Pu | P36Pi | 0.051823 | P18Pi | P22Pi | 0.035328 |
| P27Pi | P46Pi | 0.050166 | P06Pu | P07Lh | 0.034769 |
| P16Pi | P44Pi | 0.04938 | P07Lh | P46Pi | 0.033844 |
| P25Pi | P27Pi | 0.048271 | P16Pi | P26Pi | 0.033713 |
| P06Pu | P27Pi | 0.047091 | P07Lh | P25Pi | 0.031958 |
| P16Pi | P35Pi | 0.046907 | P35Pi | P46Pi | 0.031853 |
| P27Pi | P37Pi | 0.04665 | P07Lh | P37Pi | 0.031755 |
| P46Pi | P47Pi | 0.046025 | P43Pi | P47Pi | 0.03168 |
| P03Pu | P47Pi | 0.045981 | P06Pu | P39Pi | 0.030346 |
| P37Pi | P38Pi | 0.045045 | P16Pi | P36Pi | 0.028293 |
| P39Pi | P44Pi | 0.044861 | P24Pi | P38Pi | 0.027393 |
| P06Pu | P25Pi | 0.044459 | P26Pi | P37Pi | 0.024285 |
| P18Pi | P25Pi | 0.044043 | P21Pi | P36Pi | 0.024089 |
| P06Pu | P47Pi | 0.043958 | P03Pu | P35Pi | 0.023694 |
| P28Pi | P44Pi | 0.043174 | P38Pi | P46Pi | 0.023173 |
| P03Pu | P46Pi | 0.042738 | P31Pi | P44Pi | 0.020812 |
| P28Pi | P46Pi | 0.042076 | P03Pu | P21Pi | 0.019684 |
| P06Pu | P46Pi | 0.042051 | P22Pi | P39Pi | 0.0173 |
| P06Pu | P16Pi | 0.041996 | P06Pu | P37Pi | 0.015482 |
| P06Pu | P28Pi | 0.041875 | P09Pu | P21Pi | 0.015419 |
| P01Pu | P09Pu | 0.040841 | P21Pi | P25Pi | 0.01208 |
| P31Pi | P36Pi | 0.040658 | P36Pi | P47Pi | 0.011789 |
| P09Pu | P48Ci | 0.040541 | P06Pu | P44Pi | 0.011264 |
| P24Pi | P36Pi | 0.040185 | P06Pu | P36Pi | 0.010859 |
| P38Pi | P47Pi | 0.04006 | P39Pi | P46Pi | 0.008445 |
| P26Pi | P46Pi | 0.040005 | P13Lh | P21Pi | 0.0079 |
| P12Lh | P13Lh | 0.039779 | P28Pi | P37Pi | 0.007636 |
| P01Pu | P13Lh | 0.039721 | P21Pi | P22Pi | 0.007324 |
| P25Pi | P39Pi | 0.03937 | P27Pi | P43Pi | 0.007193 |
| P26Pi | P36Pi | 0.039356 | P06Pu | P22Pi | 0.006591 |
| P35Pi | P37Pi | 0.039301 | P27Pi | P44Pi | 0.0043 |
| P03Pu | P24Pi | 0.039256 | P22Pi | P27Pi | 0.00365 |
| P07Lh | P35Pi | 0.039231 | P21Pi | P38Pi | 0.003325 |
| P09Pu | P12Lh | 0.039179 | P21Pi | P43Pi | 0.003101 |
| P11Lh | P13Lh | 0.038589 | P06Pu | P21Pi | 0.002659 |
| P13Lh | P48Ci | 0.038008 | P01Pu | P03Pu | 0 |
| P31Pi | P38Pi | 0.037161 | P01Pu | P06Pu | 0 |
| P09Pu | P11Lh | 0.036165 | P01Pu | P07Lh | 0 |
| P07Lh | P38Pi | 0.035546 | P01Pu | P16Pi | 0 |

| Individual 1 | Individual 2 | Kinship Value | Individual 1 | Individual 2 | Kinship Value |
|:---:|:---:|:---:|:---:|:---:|:---:|
| P01Pu | P18Pi | 0 | P09Pu | P18Pi | 0 |
| P01Pu | P21Pi | 0 | P09Pu | P22Pi | 0 |
| P01Pu | P22Pi | 0 | P09Pu | P24Pi | 0 |
| P01Pu | P24Pi | 0 | P09Pu | P25Pi | 0 |
| P01Pu | P25Pi | 0 | P09Pu | P26Pi | 0 |
| P01Pu | P26Pi | 0 | P09Pu | P27Pi | 0 |
| P01Pu | P27Pi | 0 | P09Pu | P28Pi | 0 |
| P01Pu | P28Pi | 0 | P09Pu | P31Pi | 0 |
| P01Pu | P31Pi | 0 | P09Pu | P35Pi | 0 |
| P01Pu | P35Pi | 0 | P09Pu | P36Pi | 0 |
| P01Pu | P36Pi | 0 | P09Pu | P37Pi | 0 |
| P01Pu | P37Pi | 0 | P09Pu | P38Pi | 0 |
| P01Pu | P38Pi | 0 | P09Pu | P39Pi | 0 |
| P01Pu | P39Pi | 0 | P09Pu | P43Pi | 0 |
| P01Pu | P43Pi | 0 | P09Pu | P44Pi | 0 |
| P01Pu | P44Pi | 0 | P09Pu | P46Pi | 0 |
| P01Pu | P46Pi | 0 | P09Pu | P47Pi | 0 |
| P01Pu | P47Pi | 0 | P11Lh | P16Pi | 0 |
| P03Pu | P09Pu | 0 | P11Lh | P18Pi | 0 |
| P03Pu | P11Lh | 0 | P11Lh | P21Pi | 0 |
| P03Pu | P12Lh | 0 | P11Lh | P22Pi | 0 |
| P03Pu | P13Lh | 0 | P11Lh | P24Pi | 0 |
| P03Pu | P48Ci | 0 | P11Lh | P25Pi | 0 |
| P06Pu | P09Pu | 0 | P11Lh | P26Pi | 0 |
| P06Pu | P11Lh | 0 | P11Lh | P27Pi | 0 |
| P06Pu | P12Lh | 0 | P11Lh | P28Pi | 0 |
| P06Pu | P13Lh | 0 | P11Lh | P31Pi | 0 |
| P06Pu | P48Ci | 0 | P11Lh | P35Pi | 0 |
| P07Lh | P09Pu | 0 | P11Lh | P36Pi | 0 |
| P07Lh | P11Lh | 0 | P11Lh | P37Pi | 0 |
| P07Lh | P12Lh | 0 | P11Lh | P38Pi | 0 |
| P07Lh | P13Lh | 0 | P11Lh | P39Pi | 0 |
| P07Lh | P21Pi | 0 | P11Lh | P43Pi | 0 |
| P07Lh | P22Pi | 0 | P11Lh | P44Pi | 0 |
| P07Lh | P36Pi | 0 | P11Lh | P46Pi | 0 |
| P07Lh | P43Pi | 0 | P11Lh | P47Pi | 0 |
| P07Lh | P44Pi | 0 | P12Lh | P16Pi | 0 |
| P07Lh | P48Ci | 0 | P12Lh | P18Pi | 0 |
| P09Pu | P16Pi | 0 | P12Lh | P21Pi | 0 |

| Individual 1 | Individual 2 | Kinship Value | Individual 1 | Individual 2 | Kinship Value |
|---|---|---|---|---|---|
| P12Lh | P22Pi | 0 | P16Pi | P48Ci | 0 |
| P12Lh | P24Pi | 0 | P18Pi | P21Pi | 0 |
| P12Lh | P25Pi | 0 | P18Pi | P46Pi | 0 |
| P12Lh | P26Pi | 0 | P18Pi | P48Ci | 0 |
| P12Lh | P27Pi | 0 | P21Pi | P24Pi | 0 |
| P12Lh | P28Pi | 0 | P21Pi | P26Pi | 0 |
| P12Lh | P31Pi | 0 | P21Pi | P27Pi | 0 |
| P12Lh | P35Pi | 0 | P21Pi | P28Pi | 0 |
| P12Lh | P36Pi | 0 | P21Pi | P31Pi | 0 |
| P12Lh | P37Pi | 0 | P21Pi | P35Pi | 0 |
| P12Lh | P38Pi | 0 | P21Pi | P37Pi | 0 |
| P12Lh | P39Pi | 0 | P21Pi | P39Pi | 0 |
| P12Lh | P43Pi | 0 | P21Pi | P44Pi | 0 |
| P12Lh | P44Pi | 0 | P21Pi | P46Pi | 0 |
| P12Lh | P46Pi | 0 | P21Pi | P47Pi | 0 |
| P12Lh | P47Pi | 0 | P21Pi | P48Ci | 0 |
| P13Lh | P16Pi | 0 | P22Pi | P48Ci | 0 |
| P13Lh | P18Pi | 0 | P24Pi | P48Ci | 0 |
| P13Lh | P22Pi | 0 | P25Pi | P48Ci | 0 |
| P13Lh | P24Pi | 0 | P26Pi | P48Ci | 0 |
| P13Lh | P25Pi | 0 | P27Pi | P36Pi | 0 |
| P13Lh | P26Pi | 0 | P27Pi | P48Ci | 0 |
| P13Lh | P27Pi | 0 | P28Pi | P36Pi | 0 |
| P13Lh | P28Pi | 0 | P28Pi | P48Ci | 0 |
| P13Lh | P31Pi | 0 | P31Pi | P48Ci | 0 |
| P13Lh | P35Pi | 0 | P35Pi | P48Ci | 0 |
| P13Lh | P36Pi | 0 | P36Pi | P39Pi | 0 |
| P13Lh | P37Pi | 0 | P36Pi | P48Ci | 0 |
| P13Lh | P38Pi | 0 | P37Pi | P48Ci | 0 |
| P13Lh | P39Pi | 0 | P38Pi | P48Ci | 0 |
| P13Lh | P43Pi | 0 | P39Pi | P48Ci | 0 |
| P13Lh | P44Pi | 0 | P43Pi | P48Ci | 0 |
| P13Lh | P46Pi | 0 | P44Pi | P48Ci | 0 |
| P13Lh | P47Pi | 0 | P46Pi | P48Ci | 0 |
| P16Pi | P21Pi | 0 | P47Pi | P48Ci | 0 |