



Unraveling the interplay between demography and landscape features in shaping connectivity and diversity: Insights from the leopard cat on a subtropical island

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Abstract

Context Elucidating how demography and contemporary landscape features regulate functional connectivity is crucial to implementing effective conservation strategies. We assessed the impacts of landscape features on the genetic variation of a locally endangered carnivore, the leopard cat (*Prionailurus bengalensis*) in Taiwan.

Objectives We aim to evaluate the association between genetic structure and landscape features. We further predicted the changes in genetic diversity and suitable habitats in the future.

Methods We genotyped 184 leopard cats in western Taiwan using 12 nuclear microsatellites and a mitochondrial marker. We applied a landscape optimization procedure with two genetic distances to identify major genetic barriers and employed ecological niche modeling to predict the future distribution of the leopard cat.

Results Bayesian demographic inferences revealed a dramatic population decline for all leopard cat populations in Taiwan. Genetic clustering and resistance surface modeling supported that the population connectivity was influenced by highways and high elevation. Niche modeling indicated low temperature was one of the primary factors limiting the occurrence of leopard cats that may inhibit their movement in high elevations. We predicted the suitable habitats of leopard cats would shrink northward and towards

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higher altitudes with rugged topography in response to global warming.

Conclusions Our study provided genetic evidence that leopard cats in Taiwan had undergone a dramatic population decline that may be associated with anthropogenic impacts. We also inferred the anthropogenic linear feature compromised the connectivity and persistence of leopard cats in human-mediated landscapes. Our finding serves as a model for landscape genetic studies of island carnivores in subtropical regions.

Keywords Altitude · Climate change · Island · Landscape genetics · Road · *Prionailurus bengalensis*

Introduction

A landscape is an intricate combination of distinct habitats, and each landscape feature may impact species' distribution and ecological process (Turner 1989). Recognizing the effects of landscape features on organisms is of extreme importance for enhancing our fundamental knowledge of the environmental characteristics which shape population structure, assessing the size and boundaries of management units, and identifying populations requiring high-priority protection (Manel et al. 2003; Segelbacher et al. 2010). In urban areas, anthropogenic activities and constructions can disrupt the functional connectivity of wildlife among heterogeneous landscapes (Epps & Keyghobadi 2015; Johnson & Munshi-South 2017; Westekemper et al. 2021). Land use changes caused by human activities lead to significant habitat loss and fragmentation, making it one of the most influential threats to global wildlife and biodiversity in recent decades (Shochat et al. 2010). From a genetic perspective, highly isolated populations are more vulnerable to local extinction due to reduced fitness resulting from the accumulation of deleterious mutations and a loss of potential for adaptation to environmental change (Ellstrand & Elam 1993; Lande 1988).

Anthropogenic infrastructure, such as buildings and roads, can impede the gene flow of various species, especially of those not pre-adapted to urban environments or with limited dispersal capabilities (Fusco et al. 2021; Montgelard et al. 2014). However, some empirical studies have indicated that specific land cover types in cities can act as corridors that facilitate

gene flow and reduce population differentiation (Johnson & Munshi-South 2017; Westekemper et al. 2021). For example, roads and railways are barriers to mongoose dispersal (Grilo et al. 2009) but facilitate gene flow for foxes (Kimmig et al. 2020). In Felidae, human population density and highways disrupt cougars and tigers (Castilho et al. 2015; Yumnam et al. 2014). Anthropogenic impacts and various categories of roads also influence the movement of wildcats in Europe (Hartmann et al. 2013; Westekemper et al. 2021).

Identifying the relationship between landscape features and connectivity is crucial for effective conservation management and evaluating human impacts on endangered felids (Thatte et al. 2018; Yumnam et al. 2014). Anthropogenic climate change directly impacts wildlife behaviour, migration, reproduction, and foraging (LeDee et al. 2021; Wong & Candolin 2015). In most scenarios, rising temperatures force wildlife to migrate to new habitats at higher altitudes and latitudes (Moritz et al. 2008; Sattar et al. 2021). Artificial barriers may impede such dispersal in human-disturbed areas characterized by fragmented patches (Fusco et al. 2021). This makes it less likely for animals to disperse and adapt to climate change, especially for those with lower mobility, which can increase the risk of local extinction (LeDee et al. 2021; McLachlan et al. 2007). Therefore, conservation strategies to mitigate human-induced population decline should also consider the long-term impact of climate change.

In the present study, we investigated the landscape genetic connectivity and the demographic histories of Taiwan populations of *Prionailurus bengalensis* (Kerr 1792). *Prionailurus bengalensis* is a small carnivore widely distributed throughout Southern, Eastern, and Southeast Asia and known for its ecological adaptability (Sunquist & Sunquist 2017). Commensurate with its broad distribution, *P. bengalensis* has been classified into 12 accepted subspecies according to morphological differences, but this is currently under debate, and mitochondrial data support a reclassification into four species (Patel et al. 2017; Sunquist et al. 2009). Their adaptability is illustrated by their ability to inhabit diverse habitats, including rainforests, temperate forests, shrubland, and grassland (Sunquist & Sunquist 2017). They are also characterized by their high tolerance to human disturbance and their ability to utilize various human-dominated

habitats, including palm farms, orchards, and even semi-deserts (Rajaratnam et al. 2007; Sunquist & Sunquist 2017). Given its high adaptability and large populations, *P. bengalensis* is categorized as of Least Concern (LC) by the International Union for Conservation of Nature (IUCN), implying a low risk of extinction (Ross et al. 2015). However, regardless of its large global population size, peripheral populations on islands of the Ryukyu Archipelago (e.g., Iriomotejima Island, Tsushima Island, and Taiwan) are at higher risk of local extinction relative to populations in continental Asia (Ian 1979; Izawa et al. 2009; McCullough 1974). Given their restricted habitat and small population sizes, leopard cats on Iriomotejima Island and Tsushima Island have been listed as Critically Endangered in the Red List of the Ministry of the Environment of Japan since 2007, and those in Taiwan have been listed as Endangered under Taiwan's Wildlife Conservation Act by the Council of Agriculture since 2009. The population sizes on these three islands have greatly declined over recent decades. Increased anthropogenic pressures may be the primary threat to the persistence of leopard cats in insular habitats (Chen et al. 2016; Izawa et al. 2009).

In the past, *P. bengalensis* was documented to inhabit natural habitats at low altitudes throughout Taiwan (Chen 1956; Horikawa 1931). A fossil record discovered in southernmost Taiwan, which encompasses a far larger area than the current distribution range in western Taiwan, also indicated the presence of leopard cats in low-elevation regions since the Late Pleistocene (Chi et al. 2021). However, adverse anthropogenic impacts constituting depletion of natural habitat as well as disease transmission and attacks from invasive mammals have resulted in a severe population decline since the 1970s (Ian 1979; McCullough 1974), eliciting concerns for population sustainability (Chen et al. 2019; Pei 2008). Despite a relatively high tolerance to human disturbance and degraded habitats, leopard cats now appear to be limited to fragmented broad-leafed forests and abandoned orchards at altitudes lower than 1,500 m a.s.l in Western Taiwan (Chen et al. 2016; Pei 2008). Radiotelemetry has demonstrated that leopard cats in Taiwan alter their hunting and resting behaviours to avoid anthropogenic infrastructure and areas with high human density (van der Meer et al. 2023), suggesting that human activity may have impacted their dispersal

and connectivity. Nevertheless, the assumptions of population decline and discontinuity due to anthropogenic impacts have never been investigated using genetic analyses in Taiwan. Integrating mitochondrial and nuclear genetic information provides an opportunity to test hypotheses generated from radiotracking studies, which is critical for evaluating the conservation status of leopard cats in Taiwan and informing strategies to ensure their long-term persistence.

In landscape genetic studies, it is challenging to interpret how contemporary landscapes and historical demographic processes influence the genetic structure and gene flow due to the time lag problem (Bolliger et al. 2014; Epps & Keyghobadi 2015), which refers to the time lag between the emergence of a landscape feature and when the impacts could be genetically detectable (Epps & Keyghobadi 2015). Various molecular markers and approaches have been used to assess the effects of contemporary landscapes and historical events on genetic structures at different timescales (Epps & Keyghobadi 2015), with one such approach being to combine genetic markers displaying different mutation rates (Pérez-Espona et al. 2012). To address this issue, we combined nuclear microsatellites and mitochondrial sequences to unravel the historical and current factors influencing the genetic variation of focal species.

Based on geographic distances and potential barriers (i.e., urban areas and mountain ridges) that may disrupt the connectivity of leopard cats in Taiwan, we also defined three geographic populations, i.e., Northern, Central, and Southern groups (Fig. 1). For the contact zone between the Northern and Central groups, we assigned individuals found along the riversides of the river passing through the central regions to the Central cluster. Leopard cats in Taiwan have been documented to migrate using riversides, which suggests the possibility of a single population along the river (Pei 2008). To comprehensively evaluate the conservation status of *P. bengalensis* in Taiwan, we performed population-based analyses on pre-defined populations based on known physical boundaries to assess population admixture, genetic diversity, and gene flow. We used 12 simple sequence repeats (SSRs) and mitochondrial cytochrome b (*cytb*) sequences to tackle the following population genetic objectives of leopard cats in Taiwan:

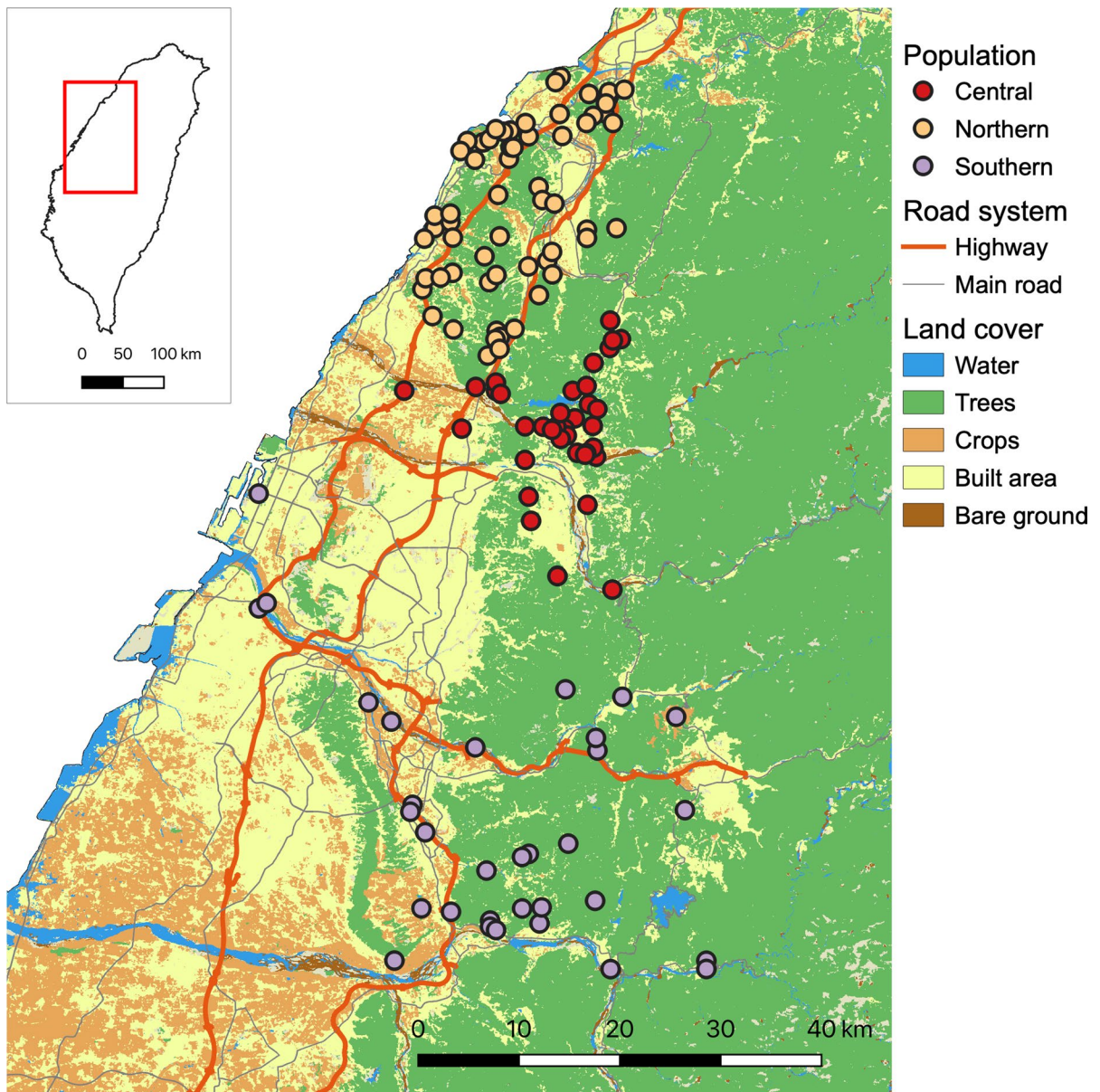


Fig. 1 Map of the sample distribution of 128 individuals with microsatellite genotyping and land cover for the study area. Different land cover and road types are indicated by colors.

Sampling sites classified according to the three pre-defined geographical populations are assigned as differently colored spots

- To depict population discontinuity, diversity, and gene flow to identify population boundaries and potential corridors.
- To describe demographic history and assess the impacts of anthropogenic and natural landscape features responsible for population discontinuities.

- To predict the influence of climate change on shifting distribution and to identify populations vulnerable to local extinction.

We present a flowchart of our data analysis to investigate these three topics in Fig. S1a. Our hypothesis is that areas with high human density,

urbanization, and atherogenic infrastructure (e.g., main roads and built-up areas) will impede the movement of *P. bengalensis* and coincide with population boundaries. On the other hand, natural habitats and undeveloped land covers like forests and grasslands will act as corridors, facilitating gene flow even in mountainous regions with complex topography.

Materials and methods

Sample collection

We sampled the known current range of leopard cats in Taiwan with two distinct sampling strategies for collecting tissues and blood. The first strategy involved fresh tissues from live leopard cats captured in Miaoli and Taichung counties from 2017–2020. The Institutional Animal Care and Use Committee (IACUC) of the National Pingtung University of Science and Technology granted permission for the capture project (Permit numbers: 106003, 106014, 107041). The second sampling scheme involved tissues collected from road-killed and rescued leopard cats from 2002–2021. We obtained 184 samples for our analyses, covering the majority of the known leopard cat distribution in Taiwan (Fig. S1b). GPS coordinates were recorded for most samples used in mtDNA analyses, and all samples used in microsatellite analyses. The methods for sample preservation and DNA purification are described in detail in the Supplementary Materials & Methods (SMM).

Genotyping and genetic diversity analyses

We genotyped leopard cats using a combination of mitochondrial *cytb* sequences and nuclear DNA microsatellites. The mitochondrial *cytb* is one of the most commonly utilized mitochondrial genes in studies of population genetics of leopard cats (Ito & Inoue-Murayama 2019; Ko et al. 2022; Teng et al. 2022). The final analyses utilized 12 previously published microsatellites (Menotti-Raymond et al. 1999). Primer information and the methods for genotyping are described in detail in the SMM. Diversity indices for microsatellites, including polymorphism information content (*PIC*), probability of identity (*PID*), expected heterozygosity (H_e), observed heterozygosity (H_o), and F_{IS} were calculated using

Cervus v3.0.7 (Kalinowski et al. 2007). Allelic richness was calculated in FSTAT v2.9.4 (Goudet 1995). We checked for the presence and frequencies of null alleles using Brookfield's method implemented in Micro-Checker v2.2.3 (Van Oosterhout et al. 2004). All microsatellites and *cytb* data genotyped in this study were deposited on Figshare (DOI: 10.6084/m9.figshare.24188403).

To precisely estimate genetic diversity based on *cytb*, we included a unique haplotype, FCN3, originating from the Central regions and identified by Tamada et al. (2008), in our dataset for subsequent analyses. This haplotype was not found in all of our genotyped individuals. Tajimas' *D* and Fu and Li's *F* and *D* were calculated to detect selection or demographic changes. All neutrality tests and diversity indices of haplotype diversity (*Hd*) and nucleotide diversity (π) were computed using DnaSP v6 (Rozas et al. 2017). The haplotype network was constructed using the median-joining method implemented in PopArt (Leigh & Bryant 2015).

Population structure and spatial genetic patterns revealed by microsatellites

We calculated pairwise F_{ST} for each population pair using Arlequin (Excoffier & Lischer 2010) to measure population diversity and differentiation. To assess the correlation between geographic areas and genetic clusters among individuals, we first applied the Bayesian algorithm in STRUCTURE v2.3.4 (Evanno et al. 2005). STRUCTURE analysis was conducted with admixture models and independent allele frequencies. Number of groupings *K* were simulated from 1 to 7 with 10 replicates for each *K*. A total of 100,000 iterations were simulated, and 25,000 iterations were set as burn-in. Geographic populations (i.e., Northern, Central, Southern) were designated priors. We ran the STRUCTURE analysis twice to check for convergence and compared the results. All results were uploaded to the CLUMPAK server (Kopelman et al. 2015) to generate consensus plots and evaluate the best *K* according to the highest ΔK value and highest $\ln(\text{Pr}(X|K))$ (Evanno et al. 2005).

We also performed Discriminant Analysis of Principal Components (DAPC) for its ability to detect subtle genetic structures without any assumption on sample source (Jombart et al. 2010). The DAPC algorithm combines PCA and DA, partitioning variance

into within-group and between-group components to maximize discrimination. We searched for the optimized number of clustering in the analysis of DAPC using the value of BIC. After determining optimized number of clustering on $K=2$ and $K=5$ from STRU CTURE and DAPC, we performed DAPC on 2, 3 and 5 clusters using the R package adegenet (Jombart 2008).

We conducted a spatial Principal Component Analysis (sPCA) to determine the spatial distribution of genetic variation using the R package adegenet (Jombart 2008). We constructed the connection network by linking the nearest 10 samples at each site. We further tested for autocorrelation of allelic frequencies using Moran's I and global and local permutation tests, with 999 replicates each. The eigenvalue of each PC axis and an eigenvalue decomposition plot were illustrated to identify spatially meaningful PC axes.

Evaluation of current gene flow and resistance surfaces

Contemporary gene flow was estimated using BayesAss (Wilson & Rannala 2003), which estimates directional migration rates over past generations using multilocus genotypes and a Bayesian approach. Mixing parameters were altered as $m=0.3$, $a=0.4$, and $f=0.5$ to tune acceptance rates between 20%–40%. Aside from estimating gene flow between populations, we adopted an estimated effective migration surfaces (EEMS) (Petkova et al. 2016) algorithm to visualize geographic regions deviating from isolation by distance (IBD), i.e., to identify areas where genetic similarity decays faster or slower than expected for a given geographic distance (known as “barriers” and “corridors”, respectively). Additional parameters for executing BayesAss and EEMS are presented in the SMM.

Inference of demographic history

DIYABC v2.1.0 (Cornuet et al. 2014) was implemented to investigate demographic history based on Approximated Bayesian Computation (ABC) using nuclear SSR markers. The analysis employed the three geographic groups previously identified. We assessed five potential scenarios of demographic history among the groups that could have produced the

current genetic variation (Fig. S2): s1, southward migration; s2, northward migration; s3-4, central origin; and s5, hybridization origin. Repeat number mutation was modelled using a generalized stepwise mutation (GSM) model, and single nucleotide indels (SNI) were allowed. One million simulations were conducted for each scenario. Logistic regression was used to compare posterior probabilities for these scenarios by examining the top 1% of the simulated datasets, based on the similarity of summary statistics to observed data. Priors for all parameters were provided in Table S1. Detailed instructions on DIYABC and model evaluation can be found in the SMM.

We further used VarEff (Nikolic & Chevalet 2014) to test for changes in effective population sizes over the past 500 years, encompassing the increase in anthropogenic activities in Taiwan (i.e., when a large number of Han Chinese immigrated to Taiwan). We set a generic mutation rate prior to 4.8×10^{-5} and adopted a GSM model with $p=0.4$, as estimated by DIYABC (see Results). Additional parameters for each population are provided in Table S2. After 10,000 burn-in iterations, we ran 10,000 steps with 100 steps per batch and 100 steps between sampled steps to avoid autocorrelation, and a total of 10,000,000 MCMC iterations were employed.

Landscape genetics

We conducted a series of regression-based analyses to test several landscape hypotheses for discovering the association between genetic distance and landscape features (Shirk et al. 2018). We applied two methods to calculate individual-based genetic distance, i.e., the Nei distance (D_{Nei}) and the Euclidean distance of the first ten axes of a principle component analysis (D_{PCA}). D_{Nei} was calculated using the R package adegenet (Jombart 2008). Detailed method can be found in the SMM. For calculating D_{PCA} , PCA and Euclidean distance were calculated using the R package vegan (Oksanen et al. 2013).

To determine the underlying landscape features shaping the genetic variation of leopard cats in Taiwan, we constructed five resistance layers (Fig. S3), the $R_{\text{elevation}}$, $R_{\text{roughness}}$, $R_{\text{human density}}$, $R_{\text{land cover}}$, and R_{road} (SMM), which are potential resistance factors for felid species or other carnivores. For example, elevation and topography roughness are barriers to mon-goose (Barros et al. 2017); anthropogenic factors such

as human density and human-dominant landcover may hinder the movement of wildcats (Hartmann et al. 2013); and a variety of roads are significant barriers to wildcats in Europe (Westekemper et al. 2021).

Developing resistance surfaces is challenging due to the difficulty in assigning specific values to landscape features (Peterman et al. 2019; Zeller et al. 2012). To overcome this, genetic data can be used to optimize resistance layers (Peterman et al. 2019). We applied the genetic algorithm (GA) implemented in the R package ResistanceGA (Peterman 2018) for developing resistance layers (SMM). We optimized each resistance layer using a single surface optimization procedure with the function `SS_optim()` in the R package ResistanceGA. All continuous and categorical layers were optimized with commute-time distance calculated in the R package `gdistance` (van Etten 2017), equivalent to circuit-theory-based distance. The maximum resistance of each layer was set to 500, resistance values were optimized utilizing Akaike information criterion values (AIC) as criteria, and other default parameters were adopted. Each resistance layer was optimized with two independent runs using D_{Nei} or D_{PCA} as a dependent variable to check for convergence. In addition to optimizing a single surface separately, we employed multiple surface optimization using ResistanceGA's wrapper function `all_comb()` to optimize all possible combinations automatically.

After optimizing single and all possible multiple resistance models, we conducted model comparison using two independent approaches: the Maximum Likelihood Population Effects mixed effects model (MLPE) (Clarke et al. 2002) and Reciprocal Causal Modeling (RCM) (Cushman et al. 2013), both of which offer high accuracy for selecting proper landscape features affecting genetic differentiation under different conditions (Peterman et al. 2019). For MLPE, model performance was assessed with corrected Akaike information criterion values (AICc), and models with $\Delta AICc < 2$ are considered to perform equally well. RCM compares the Mantel R of all possible model combinations of all resistance layers to find the best model for describing genetic differentiation. The model of geographic distance was also added as a null model to the computation of the RCM algorithm. Detailed methods for performing model

comparison are described in the SMM. We also applied the `resist_boot()` function in ResistanceGA to conduct a bootstrap analysis on (1) all possible combinations of resistance models and (2) single-surface resistance models only.

Species distribution modelling (sdm) and potential diversity loss to future climate change

To incorporate the influence of climate change and assess the potential habitats under different climate scenarios in the future, we employed a species distribution modelling (sdm) approach to construct niche models and project models to current and future (in 2070) climate conditions. Given the lack of reasonable predictions for road and landcover alterations in the future, we only used 19 bioclimatic variables related to temperature and precipitation, which are suitable for predicting potential distribution areas of wild mammals (Holzmann et al. 2015). The 19 bioclimatic variables were downloaded from WorldClim2 (Fick & Hijmans 2017), with a resolution of 30 arc-sec (about 1 km² per cell). The average of three global climate models, i.e., CCSM4, MIROC-ESM, and MIROC5, in 2070 was implemented under two contrasting representative concentration pathways (RCPs), a low-emission model (RCP2.6) and a high-emission model (RCP8.5) from CMIP5 (Taylor et al. 2012) for prediction. We conducted an ensemble approach that makes predictions based on combining six methods, including `maxent`, `glm`, `svm`, `gam`, `mda`, and `mlp`, for model construction using the R package `sdm` (Naimi & Araújo 2016). Details are described in the SMM. Area under the receiver-operator curve (AUC) and true skill statistic (TSS) were used to evaluate model performance.

To simulate potential diversity loss associated with climate change, we calculated genetic diversity indices, including H_e , allelic richness (AR), and F_{IS} , under current habitat and climate in 2070 for individual samples overlapping with habitat suitability > 0.5 and > 0.75 on the prediction maps, respectively. A Kruskal Wallis test (Hollander et al. 2013) was performed to determine significant differences in diversity among groups using the R package `stats` (R Core Team 2013).

Results

Genetic diversity of microsatellite and mitochondrial markers

We genotyped 128 individuals for microsatellites and sequenced 113 individuals for mitochondrial *cytb*, including 57 samples at both microsatellite genotyping and *cytb* sequencing in the analyses (Table S3). In our datasets (including mtDNA and microsatellites) that mainly collected from road-killed samples, 72 were female and 92 were male. For pre-classified geographic groups, 79, 47, and 43 samples were collected from northern, central, and southern regions respectively. Overall, our collection spans almost all known distributions of leopard cats in Taiwan. All 12 microsatellite loci were polymorphic (mean PIC=0.51, ranging from 0.11–0.75; Table S4) and demonstrated a low frequency of null alleles (mean=0.03, ranging from 0–0.09). All genetic statistics were provided in Table S4.

The overall genetic diversity for *cytb* was low ($H_d=0.393$; $\pi=0.00036$). We detected four *cytb* haplotypes, with only 1–2 basepair (bp) differences among haplotypes (Fig. 2d), including one haplotype (FCN3) that was not detected among our tissue samples. No clear geographic pattern to haplotype occurrences was found, with the two predominant haplotypes (PbTaiC1 and PbTaiC2) displaying comparable proportions across all geographic regions (Fig. 2d). All neutrality tests, including Tajimas' D , as well as Fu and Li's F and D , were non-significant ($p>0.1$), indicating no selection or abrupt changes in population size.

Population structure and gene flow

The three geographic populations classified a priori according to our assumptions demonstrated moderate genetic differentiation (pairwise F_{ST} , Fig. 2c), with all pairwise differentiation being statistically significant ($p<0.05$; Fig. 2c). The highest value was observed between Northern and Southern ($F_{ST}=0.12$) and the lowest between Central and Northern ($F_{ST}=0.06$) populations. All populations displayed comparable expected heterozygosity (H_e), ranging from 0.53–0.54, and a low F_{IS} value of less than 0.1. The genetic statistics for each population are provided in Table S4.

The two independent STRUCTURE analyses suggested similar results, indicating a best K of 2 (Fig. S4a) based on ΔK , which roughly separated the Northern group from the other two populations, and samples with admixed genetic composition were found in all populations (Fig. 2a). Based on $K=3$, the Central and Southern populations were further differentiated with a certain level of admixture. The results of the DAPC supported a similar pattern with STRUCTURE, indicating an overall North–South differentiation and mixing in the Central region (Fig. 2a). Results of other numbers of K of STRUCTURE and clustering numbers of DAPC are provided in Fig. S5.

Regarding these spatial relationships, the statistically significant Morans' I ($p<0.05$) and global structure ($p<0.05$) indicated positive spatial autocorrelation. The lagged principal scores from axis 1 also support a North–South division of the genetic structure (Fig. 2b; Fig. S6), following our STRUCTURE and DAPC findings.

BayesAss indicated significant population migration, with 2 to 6% of individuals emigrating per generation, even between the Northern and Southern populations (Table S5). From the EEMS plot, we observed two noticeable barriers with reduced gene flow, corresponding to highways and mountainous areas (Fig. 3; Fig. S7). The first barrier, reflecting North–South highways and mountain ridges, separates the Northern and Central populations. The second barrier, represented by a highway and unfenced main roads extending from the west coast to mountains in the (south)east, separates the Southern population from the other populations. One obvious genetic diversity hotspot encompassing the Central population was revealed by EEMS, which harboured a highly proportional admixed genetic composition (based on the STRUCTURE analysis) compared to other regions (Fig. 3). Last, two genetic diversity coldspots were located at the northernmost and southernmost borders of the current distribution of the leopard cat in Taiwan (Fig. 3).

Demographic history

The observed data set in DIYABC was located in the middle of simulation priors and a combination of posterior parameters using PCA, indicating a good fit of the model (Fig. S8). Among the tested scenarios, scenario 5 (s5; an admixed origin of the

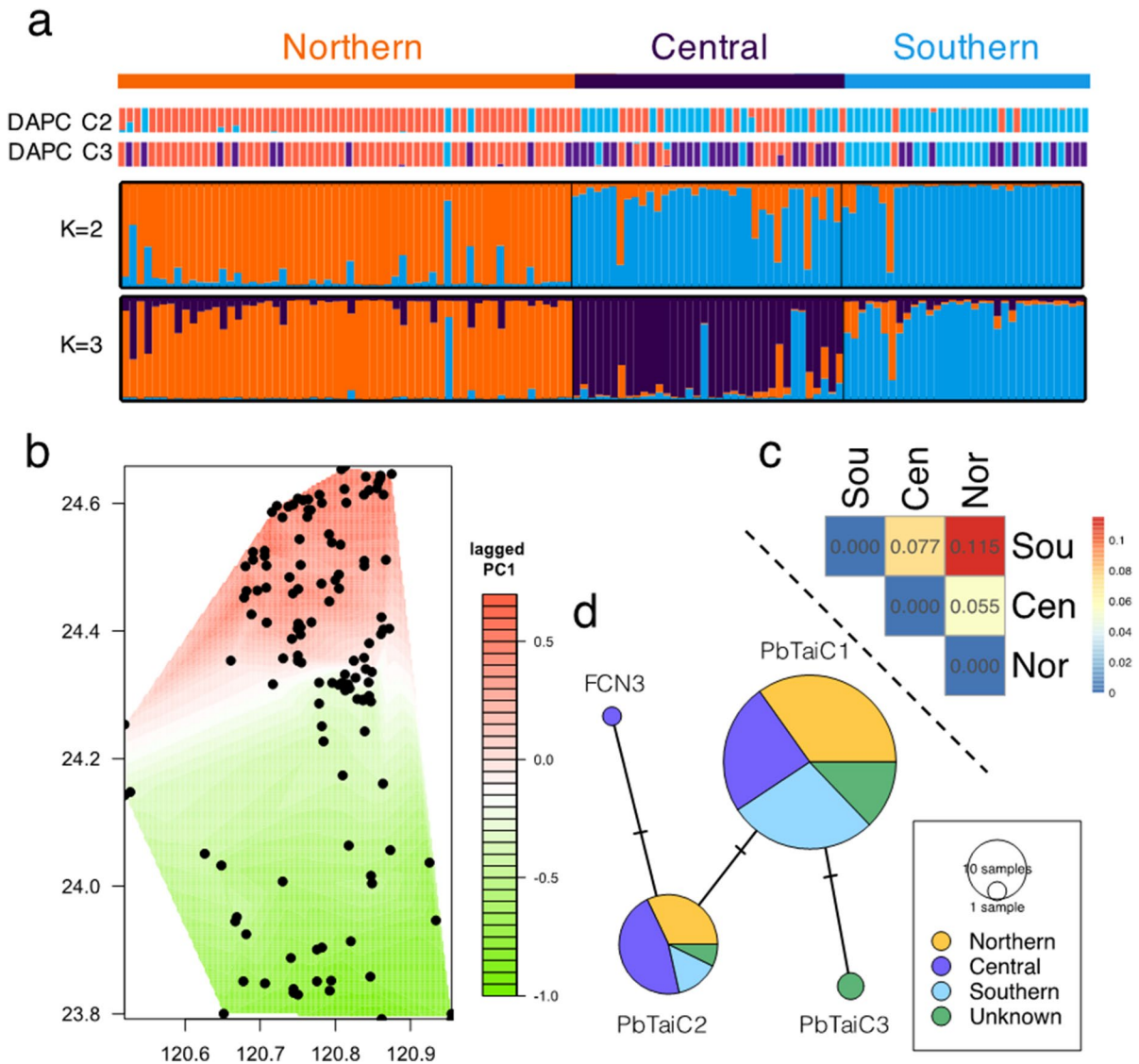


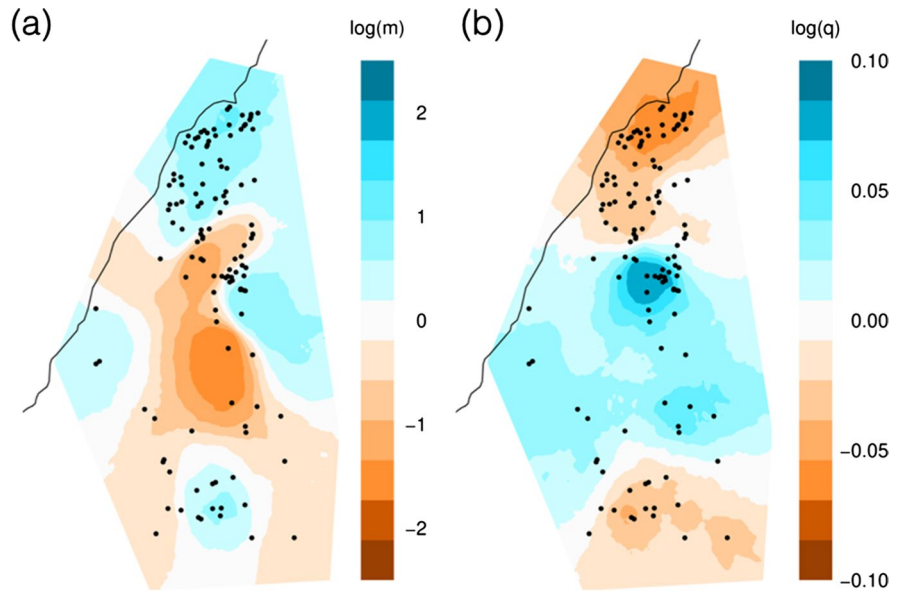
Fig. 2 Genetic differentiation revealed by microsatellites and mitochondrial sequences. **(a)** Results of DAPC and STRUCTURE. Colors represent the assignment probabilities of individuals to certain cluster memberships. DAPC results (top) are shown with values of $C=2$ and $C=3$. Plots from STRUCTURE with values of $K=2$ to 3 show that the genetic structure is roughly coincident with geographic populations. **(b)** Results of the sPCA that visualized using lagged principal scores from

the first global axis with specific interpolated coordinates. Sampling sites added to the map. **(c)** Pairwise F_{ST} between populations. All values are significant ($p < 0.05$). **(d)** Median-joining haplotype network of *cytb* haplotypes (1,140 bp) and assigned populations. Colors of haplotypes represent the geographic groups; samples without geographic information were represented as “Unknown”; sizes of dots denote the number of specific haplotypes

Central population) was the most supported based on the highest logistic regression estimate of posterior probability, with no overlap of the 95% confidence interval with other scenarios ($P=0.312$, 95% CI 0.286–0.337; Table S6). In this scenario,

the divergence of the Southern and Northern populations (td) occurred 813 years ago (95% CI: 257–5010 years ago), and the Central population was generated 208 years ago (95% CI 40.6–969) through interbreeding between the Northern and

Fig. 3 EEMS—estimated effective migration and diversity rates. (a) Interpolated surface of the mean migration rate m . Negative values in red indicate reduced gene flow (barriers), whereas positive values in blue represent facilitated gene flow (corridors). (b) Interpolated surface of the mean diversity q depicting diversity across the study area. Negative values in red indicate low diversity and positive values in blue denote higher-than-expected diversity



Southern populations. The interbreeding involved more immigrants from the Northern population (60%) than the Southern population (40%; Table 1).

From the demographic histories inferred from VarEff, all three populations showed a steady decline in effective population sizes over the last 500 years, followed by a sudden and steep drop in population size approximately 150–300 years ago, indicating that the populations underwent genetic bottlenecks during this period (Fig. S9).

Landscape genetics

Our optimization process on five single resistance layers revealed convergence on the same predictor ranking in two runs using AICc. We found that elevation and roads was the best two models (Table 2). Elevation also received the highest support as the top-ranked single-surface model across all bootstrap iterations (Table S7). For the elevation model, optimized resistance was positively correlated with elevation, dramatically increasing at low elevation and reaching a plateau at approximately 1,500 m a.s.l. Although roughness was positively associated with resistance,

Table 1 The results of estimated parameters from the best-supported demographic scenario (s5). $Ne_{Northern}$, effective population size of Northern population; $Ne_{southern}$, effective population size of Southern population; $Ne_{Central}$, effective population size of Central population; $t1$, time of secondary

contact between Southern and Northern populations (in years); ra , relative genetic contribution from Southern population; $t2$, time of divergence between Southern and Northern populations; μ , mutation rate; p , parameter of the geometric distribution

Parameter	Mean	Median	Mode	95% CI
$Ne_{Northern}$	6400	6530	7130	2380–9690
$Ne_{Southern}$	7680	7980	8390	3690–9880
$Ne_{Central}$	4600	4310	3250	851–9420
$t1$	346	292	208	40.6–9420
ra	0.456	0.44	0.388	0.0554–0.918
$t2$	1480	1160	813	257–5010
μ	0.0000645	0.0000527	0.0000468	0.0000229–0.000179
p	0.559	0.537	0.444	0.261–0.929

Table 2 Results of the averaged single surface ResistanceGA optimization

Surface	k	AIC	AIC _C	R ² _m	R ² _C	LL
(a) Nei distance (D _{Nei})						
Elevation	4	-8911.031	-8910.706	0.143	0.379	4459.515
Road	4	-8892.456	-8892.131	0.135	0.375	4450.228
Roughness	4	-8878.307	-8877.981	0.136	0.368	4443.153
Distance	2	-8877.835	-8877.739	0.107	0.346	4440.917
Human density	4	-8874.788	-8874.463	0.11	0.346	4441.394
Land cove	7	-8864.792	-8863.859	0.108	0.357	4439.396
Null	1	-8053.686	-8053.654	0	0.254	4027.843
(b) Ten-axes-based PCA Euclidean distance (D _{PCA})						
Elevation	4	8538.546	8538.871	0.113	0.367	-4265.273
Road	4	8551.904	8552.23	0.03	0.347	-4271.95
Roughness	2	8565.696	8565.792	0.051	0.3	-4280.848
Distance	4	8567.37	8567.695	0.08	0.322	-4279.685
Human density	4	8569.512	8569.837	0.05	0.3	-4280.756
Land cove	7	8575.589	8576.523	0.06	0.307	-4280.795
Null	1	8945.518	8945.55	0	0.26	-4471.759

Two genetic distance (a-b) and five environmental predictors. The results of bootstrap analysis for the single resistance models are presented in Table S11. k, number of parameters; AICc, corrected AIC; R²_m, marginal R²; R²_C, conditional R²; LL, log likelihood

it only exhibited a significant increase at high roughness (Fig. S10). Regarding road layer optimization, the category of highways displayed the highest resistance, acting as barriers to gene flow, whereas the category of main roads exhibited the lowest resistance compared to the patches category, denoting corridors for *P. bengalensis* (Fig. 4).

After optimization using all possible combinations of resistance layers, we generated 21 models for each genetic distance. Model comparisons using AICc did not converge on the same results for the D_{Nei} and D_{PCA} distances. Elevation+roads was identified as the best multi-surface model using D_{PCA} distance, whereas elevation+roughness was the best-supported model using D_{Nei} (Table S8). Considering the small delta AICc (<2) and comparable marginal R² of the second-best model, we believe the performance of the elevation+roughness+roads model is as good as the top model using D_{Nei}. The bootstrap analysis using D_{Nei} supports the elevation+roughness model as being the best, followed by the elevation+roads model. In contrast, bootstrap analysis using D_{PCA} distance indicated the elevation model as the best, followed by the elevation+roads model (Table S9). Finally, model comparison using RCM also generated inconsistent results between genetic distances. In this

case, the elevation+roughness+roads model was superior to all alternative models using D_{Nei}, whereas the Landuse+human density model was selected as the best using D_{PCA} (Fig. S11). Overall, elevation was the most significant single variable, regardless of the genetic distance used. Models including elevation and roads exhibited the highest support according to AICc and the second highest support based on bootstrap analysis, using both types of genetic distance.

Prediction of suitable habitats and diversity loss under climate change

After eliminating highly correlated factors, we implemented six factors to construct species distribution models: three precipitation-related (BIO6, BIO8, BIO9) and three temperature-related (BIO13, BIO14, BIO19) variables. Sixty models were generated with high predictive accuracies with an AUC of 0.97 (SD=0.005). The response curves and relative importance (Fig. 5a-b) showed that the occurrence of *P. bengalensis* was fundamentally limited by the precipitation of the driest month (BIO14), the precipitation of the coldest quarter (BIO19), and the mean temperature of the wettest quarter (BIO8). Using the consensus predictions (TSS=0.91, SD=0.032),

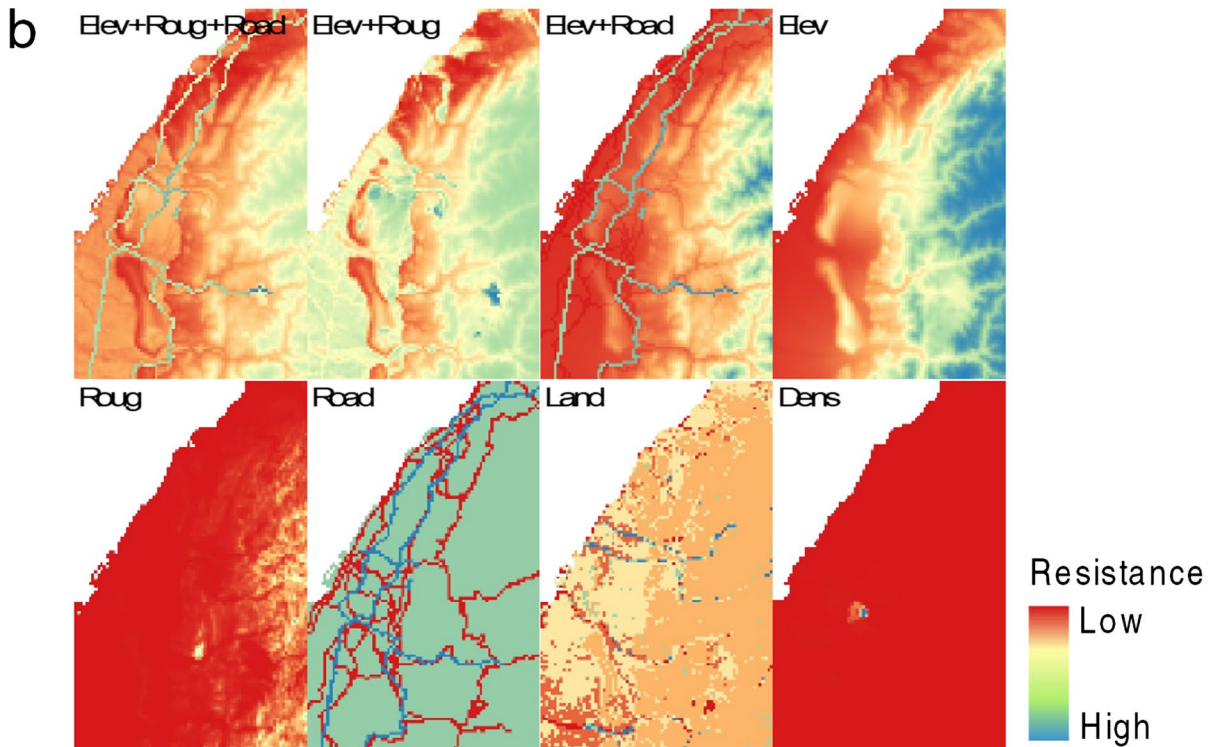
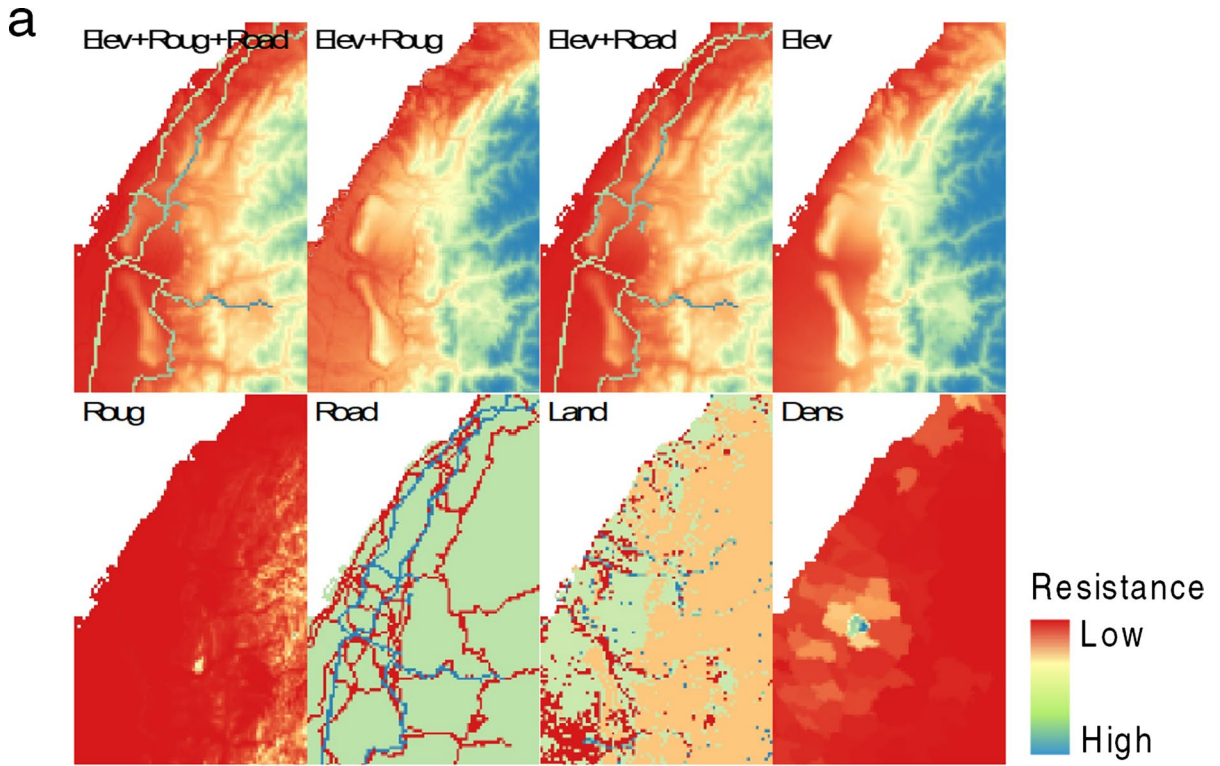


Fig. 4 Optimized resistance surfaces using two genetic distances, including the top three best-supported multi-surface models and five single models. The color gradient on maps represents resistance values from low (red) to high (blue). (a) Models optimized using ten-axes-based PCA Euclidean distance (D_{PCA}). (b) Models optimized using Nei distance (D_{Nei}). Elev, elevation; Roug, roughness; Road, roads; Land, land cover; Dens, human density

suitable habitat for *P. bengalensis* will have shrunk by 2070 under both emission models, with the predicted contraction being more pronounced according to the model of increased global warming (RCP 8.5) (Fig. 5c). In 2070, habitats at lower altitudes and southern mountainous regions are less suitable. A reduction in highly suitable habitats further to the north and in rugged mountainous areas is also predicted. A higher proportion of individuals from the northern and central clusters overlap with highly suitable habitats ($P > 0.75$) and moderately suitable habitats ($P > 0.5$) compared to southern individuals (Table S10). More than 95% of northern and central samples overlap with moderately suitable habitats in both climate change scenarios in the future, whereas only 19% of southern samples fall into moderately suitable habitats (Table S10). There were no significant differences in any of the diversity indices among groups (all with $P > 0.05$) according to the two values of habitat suitability we used (Fig. 5d).

Discussion

As the only extant felids in Taiwan, leopard cats may play a crucial role as apex predators, contributing to ecosystem stability and trophic cascades (Pace et al. 1999; Terborgh et al. 1999). The conservation of leopard cats may also benefit the protection of local habitats and other species as an umbrella species. Our study is the first to focus on the individual-based landscape genetics of *P. bengalensis* on a subtropical island, aiming to identify fine-scale genetic boundaries among landscapes (Kierepka & Latch 2015). In the present study, we aimed to characterize the spatial genetic structure and demographic history of leopard cats in Taiwan. We identified an interbreeding event that generated a genetic diversity hotspot. We also described a consistent decline in population size that may be associated with increased anthropogenic impacts in Taiwan. The current population structure

may indicate which features seem to be barriers to gene flow (e.g. highways, topographical roughness), but natural habitats and main roads at lower elevations may act as movement corridors (Fig. 4). Additionally, we anticipate that climate change will amplify habitat fragmentation in the near future. Moreover, we demonstrated that road-killed samples with a sufficient sample size gathered over an appropriate study area can provide insights into ecological processes and landscape connectivity. Although the collection may take a long time to gather enough samples for performing rigid statistical analyses.

Landscape and demographic history shape the current genetic structure of leopard cats in Taiwan

Our findings supported a correlation between genetic differentiation and landscape features. Whereas our nuclear markers revealed spatial clusters, mtDNA haplotypes exhibited no spatial genetic pattern. Together with the absence of signal of selection on *cytb* sequences, suggesting *cytb* sequences alone may not be able to provide biologically meaningful signals for our focal species (Fig. 2d). In other mammalian studies using mtDNA, control regions (CR or D-loop) may also be a more suitable marker to reveal the genetic variation at the population level because it may have more informative sites and higher diversity (Alter & Palumbi 2009; Bickham et al. 1996; Tamada et al. 2005). However, the special genetic analyses using microsatellites revealed that the predefined populations (i.e., Northern, Central, Southern) demonstrated an obvious and significant genetic differentiation, underlying our hypothesis about the potential barriers of anthropogenic features (e.g., highways) and mountainous ridges to the movements of leopard cats in Taiwan.

Although our mtDNA data could not provide insights into historical events, by applying a variety of approaches to microsatellites, combining landscape genetic analyses and coalescent methods (Epps & Keyghobadi 2015), we revealed associations between current landscape features and population structures with historical evolutionary events also contributing to the genetic profile of leopard cats.

Our analysis of colonization history revealed a divergence between the Northern and Southern populations 800 years ago, followed by a secondary contact 200 years ago. We infer that this secondary contact generated the genetic diversity hotspot and

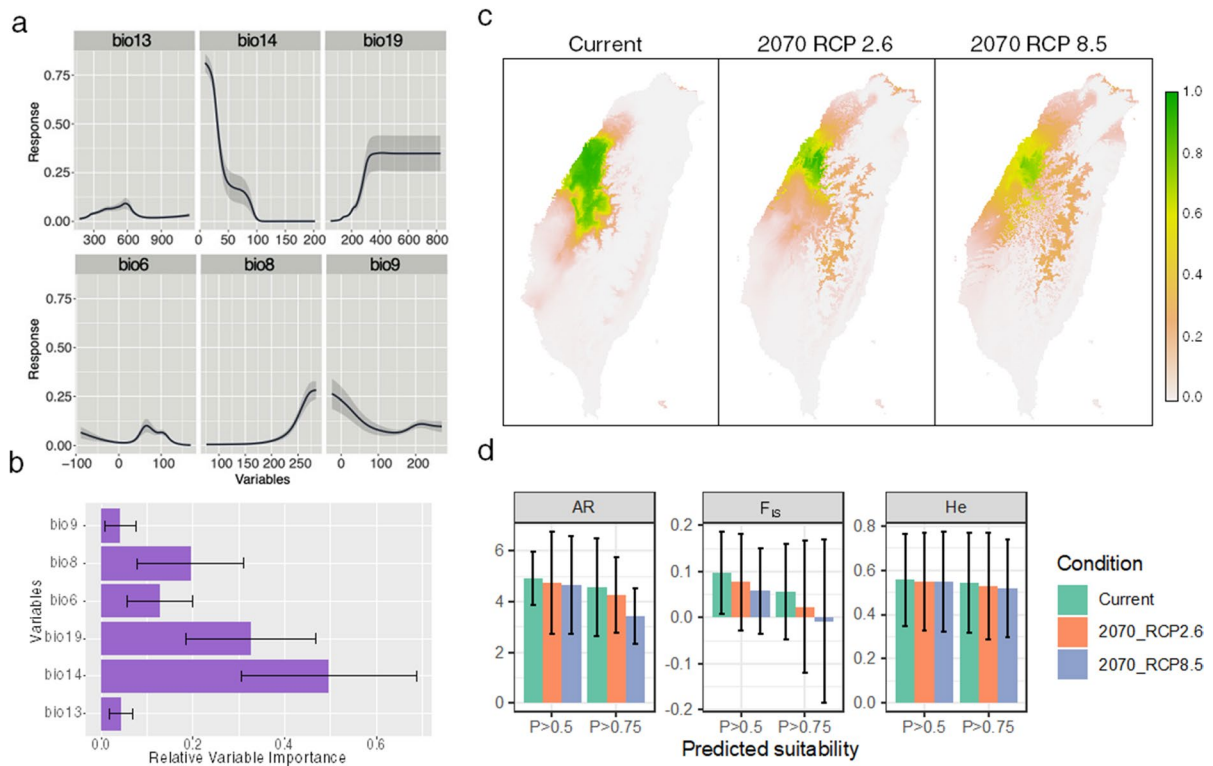


Fig. 5 Distribution models representing areas of climate suitability and predicted diversity indices over time. (a) Values of the response curves shown are averages over 60 runs; grey margins show standard deviation over all replicates. (b) Relative importance that evaluated using AUC, with standard deviation calculated for each variable. (c) Relative suitability under current climate and the two emission models (i.e., RCP 2.6 and RCP 8.5) in 2070. The color spectrum represents suitability

on the maps, from areas with low (0) to high (1) values. (d) Comparison of diversity indices over time. The predictions are grouped by different data subsets using sample locations overlapping with moderately suitable ($P > 0.5$) and highly suitable ($P > 0.75$) habitats. The error bars denote the standard deviation for each index. *AR*, allelic richness; F_{IS} , inbreeding coefficient; H_e , expected heterozygosity

the pattern of admixture observed in the Central population from the previously isolated populations. For example, human activities and the development of natural habitats provide opportunities for migration and result in secondary contacts (Grabenstein & Taylor 2018). Similarly, the intended development and population growth of humans may force the historically differentiated Northern and Southern populations to contact again in the region of the current Central population within the past 200 years.

We found evidence of bottleneck events in the past 200 years in all three populations, coinciding with rapid human development in Western Taiwan (Barclay 2015; Wu 2004). Although the exact cause of this change in demographic history remains unclear, historical climate fluctuations and human activities are potential contributing factors. Dramatic

demographic changes of mammals in other regions have been attributed to climate variations and human disturbances (Hsiung & Sunstein 2006). Habitat loss, deforestation, and changes in land cover for residential or commercial use may deteriorate the persistence of several species in Taiwan, including leopard cats (Chang et al. 2012; Chen et al. 2016; Hwang et al. 2010). Consequently, the drastic population decline event followed by human-mediated migration may be attributed to the distribution of leopard cats nowadays that strictly limited to few regions in Western Taiwan.

An alternative explanation for the hybridization event occurring 200 years ago could be attributed to a 7-year restriction (1895–1902) imposed on Han people, halting forest development at the outset of the Japanese colony in Taiwan (Ye 2018). Historically, a significant portion of natural forests in western

Taiwan has been cleared by Han settlers since the seventeenth century (Barclay 2015; Wu 2004). These degraded forests may have acted as barriers, isolating leopard cats from central and southern populations. During the restriction period at the beginning of the Japanese colony, reduced human activities in the western Taiwanese forests may have allowed previously isolated leopard cat populations to come into contact once again. However, as no discernible population growth was identified during the hybridization events, we suggest that this secondary contact might imply geographic expansion rather than population growth, as previous studies have indicated that geographic expansion and population expansion may not always be correlated (Excoffier et al. 2009; Williams & Giddings 1984). Furthermore, some studies have suggested that hybridization events can occur despite continuous declines in overall population sizes (Pellegrino et al. 2015; Shi et al. 2023). In our study, even if restrictions 200 years ago prevented humans from colonizing forests for about seven years, this time-frame may not have been sufficient for the forest system to recover. Conversely, degraded forests may not support population growth for leopard cats but could function as corridors facilitating migration and gene flow between southern and northern populations during the early stages of forest restoration.

Gene flow in a heterogeneous matrix with human disturbance

Despite inconsistencies between results generated using PCA-derived or Nei's genetic distance and ResistanceGA and RCM approach, elevation and roads appeared among the most critical landscape features to shape landscape genetic connectivity in Taiwanese leopard cats. Contrary to our expectations, resistance to movement increased with elevation, even though natural forests dominate mountainous regions. This outcome may be explained by a preference for forest edges, which provide easy access to refuge in forests and prey in agricultural landscapes for leopard cats and European wildcats (Hartmann et al. 2013; Rajaratnam et al. 2007). Tracking studies conducted in Taiwan also suggested that leopard cats prefer densely vegetated natural habitats and avoid areas with human activities (van der Meer et al. 2023). An alternative explanation can be derived from the concept of the energy landscape, whereby

landscape complexity influences animals to adopt the most energetically efficient movement patterns (Wilson et al. 2012). We observed a positive correlation between resistance and roughness (a proxy of topological complexity), but resistance only increased significantly at high roughness levels and higher elevations (> 1,000 m). Thus, landscape complexity alone does not fully explain why elevation positively correlated with resistance at lower elevations (< 1,000 m). We propose a third potential explanation based on the typical physiological intolerances to large temperature variations of tropical organisms (Janzen 1967). We found that the distribution of leopard cats in Taiwan was negatively correlated with minimum temperature (Fig. 5a), indicating that low temperatures may limit their expansion and movement. Unlike their relatives in temperate areas, tropical and subtropical leopard cats are typically restricted to lowlands (Rajaratnam et al. 2007; Ross et al. 2015). Moreover, elevation as a significant barrier has been reported for diverse taxa in tropical regions (Monteiro et al. 2019). It is noteworthy that using RCM and D_{PCA} genetic distance, the best-supported model included Land use and Human density, suggesting the putative influences of anthropogenic factors may also be a profound factor that shapes the movement of leopard cats, as revealed in the studies of other felids (Hartmann et al. 2013).

Linear features such as paved roads and highways are artificial structures known to have a significant impact on gene flow in many species (Clark et al. 2010; Kuehn et al. 2007; Robinson et al. 2012), and highways have been identified as physical barriers that impede movement and lead to a decline in genetic diversity (Epps & Keyghobadi 2015). Huge artificial linear features may also act as behavioural barriers (Kimmig et al. 2020; Laundré et al. 2010). Similarly, we inferred that the avoidance of highways of leopard cats in Taiwan may be driven by a perceived risk associated with high-speed traffic and strong illumination. In contrast, we found that unfenced roads presented low resistance, serving as corridors that facilitate movement. Such roads are often located in areas with lower human activity and traffic volume than highways. In mountainous regions, roads are typically situated in valleys at lower elevations and are surrounded by natural forests, making them suitable for leopard cat movement (Chen et al. 2016; Pei 2008). Although vehicle collisions pose a significant

mortality risk for leopard cats in Taiwan (at least 50 road-kills recorded between 2012 and 2017; Chen et al. 2019), they may still use roads as corridors to avoid other human activity areas. In our data collection, only 12% (18/152) of road-killed samples with GPS coordinates were collected from highways (Table S3). Similar patterns have been observed for foxes, which prefer sites of low human density and activity rather than completely preventing human constructions (Adkins & Stott 1998). These findings highlight how different linear landscape features influence connectivity and can contribute to our understanding of dispersal processes in vagile carnivores.

Conservation implications

Our genetic study complements previous ecological studies revealing that direct and indirect impacts of anthropogenic features have compromised the sustainability of Taiwanese leopard cat populations. Although there is currently no evidence of severe genetic drift that may significantly affect the genetic diversity among leopard cat populations in Taiwan, human-mediated landscape features have substantially contributed to genetic differentiation among its populations. Despite no prospect of significant loss of diversity in the near future, our species distribution models predict that climate change may further endanger the viability and connectivity of Taiwanese populations, restricting suitable habitats to northern and mountainous regions with rugged topography, which the high elevation and complex topography are supposed to act as profound resistance to movement and gene flow (Fig. 5c; Fig. S10). The mitochondrial marker revealed extremely low genetic diversity in the leopard cat populations of Taiwan among related populations in Far East Asia (Ito & Inoue-Murayama 2019; Ko et al. 2022; Teng et al. 2022). Moreover, the distinct Taiwanese haplotypes indicate limited gene flow between peripheral island habitats and continental core populations (Patel et al. 2017; Tamada et al. 2008). The evolutionary potential to adapt to changing environments depends on the balance between genetic variation and drift (Brown 1984; Holderegger et al. 2006). However, the low genetic variation resulting from founder effects and lack of new genetic components increases the risk of local extinctions

in island populations facing extreme climate change (Johnson et al. 2000; Wood et al. 2017).

The critical conservation management actions for Taiwanese leopard cats should focus on maintaining neutral and adaptive genetic diversity, population fitness, and evolutionary potential in the face of habitat fragmentation and impending climate alterations. Compared to the northern and central populations, conservation priority should also be placed on the southern population. This is because most of the currently suitable habitats that overlap with the southern population are predicted to become extinct in the future due to climate change (Table S10). Efforts should be made to reduce adverse human activities that drive genetic differentiation in the island's leopard cats, such as establishing corridors to facilitate gene flow between fragmented habitats. Protected areas can be designated as areas of high dispersal potential to minimize disturbance from human activities and predation by free-roaming domestic dogs. To mitigate fragmentation and reduce the risk of road collisions, artificial underground or aboveground corridors can be implemented along highways in areas with a high population density of leopard cats (Beier & Noss 1998). Lastly, to counteract the negative effects of drift and inbreeding caused by isolation and climate change, establishing corridors connecting southern and northern areas should be a high priority to prevent the loss of genetic diversity and reduce the risk of local extinctions. Our study provides valuable insights into how landscape features shape the evolutionary processes and future distribution of a locally endangered felid on a subtropical island, offering fundamental ecological information for its conservation.

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Author contributions Y.T.J. designed and supervised the project. J.C.P., Y.H.L., D.J.L., M.T.C., and P.K.J.C. collected the samples and provided valuable feedback. C.H. and L.W. performed laboratory experiments and analyzed the mitochondrial data. P.W.S. performed statistical analyses of demographic history, landscape genetics, and niche modeling using the input from C.H. and L.W. P.W.S. and C.H. drafted the manuscript. P.C.L. assisted with data interpretation, provided valuable feedback, and revised the manuscript. All authors have read and approved the final version of the manuscript.

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Data availability DNA sequences are available at Supporting Information and NCBI GenBank (PbTaiC1: accession number OR126345; PbTaiC2: accession number OR126346; PbTaiC3: accession number OR126347). Sampling information and SSR genotyping data are in the Supporting Information.

Declarations

Competing interests The authors have no relevant financial or non-financial interests to disclose.

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