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Genetic diversity in clustered colonies of an Antarctic marine mesopredator: a role for habitat quality?

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Abstract: Genetic structure may be highly variable across seabird species, and particularly among those that are distributed over large geographical areas. The Adélie penguin (*Pygoscelis adeliae*) is a numerically dominant Antarctic seabird that is considered to be a key species in coastal ecosystems. Since the Last Glacial Maximum, penguin colonization of the Antarctic coastline occurred at varying geographical and temporal scales, contributing to an incomplete understanding of how modern colonies relate to each other at local or regional scales. We assessed the population genetic structure of Adélie penguins ($n = 86$ individuals) from three adjacent colonies along the Victoria Land coast using molecular genetic markers (i.e. seven microsatellite loci isolated through next-generation sequencing). Our results indicate meta-population dynamics and possibly relationships with habitat quality. A generally low genetic diversity (Nei's index: 0.322–0.667) was observed within each colony, in contrast to significant genetic heterogeneity among colonies (pairwise $F_{ST} = 0.071$–0.148), indicating that populations were genetically structured. Accordingly, an assignment test correctly placed individuals within the respective colonies from which they were sampled. The presence of inter-colony genetic differentiation contrasts with previous studies on this species that showed a lack of genetic structure, possibly due to higher juvenile or adult dispersal. Our sampled colonies were not panmictic and suggest a lower migration rate, which may reflect relatively stable environmental conditions in the Ross Sea compared to other regions of Antarctica, where the ocean climate is warming.

Introduction

Ocean currents and oceanographic barriers may influence the dispersal behaviour of marine predators, therefore affecting gene flow and facilitating biogeographical breaks among populations (Vianna *et al.* 2017). Natal philopatry may play a major role in shaping population demographics and the genetic structure of marine seabirds (Milot *et al.* 2008). Return to natal localities may have several advantages (e.g. population 'knowledge' about food resources and predators), but the occurrence of philopatry can be influenced by the degree of environmental variability or population pressures (Coulson 2016).

Given its geographical isolation including regional oceanographic barriers, populations of Antarctic organisms may be locally adapted and therefore show genetic structuring (Vianna *et al.* 2017). The Antarctic Polar Front and the Antarctic Circumpolar Current are major driving forces that influence species distribution, serving as barriers or facilitators to animal movements in both north-south and clockwise directions, respectively (Vianna *et al.* 2017). Vertebrate distribution and colonization of Antarctica have been shaped by cycles of glacial and interglacial periods (Cole *et al.* 2019). During the Holocene (the last 10 kya), the distribution and occupancy rates of marine bird and mammal species sensitive to changes in coastal glacial and sea-ice conditions have been influenced by ice-sheet advances and retreats, as well as by pack-ice cover, fast ice and polynya extension (Mezgec *et al.* 2017). For instance, populations of the Adélie penguin (*Pygoscelis*

adeliae) are circumpolar and exhibit two distinct and divergent mitochondrial lineages (A, 'Antarctica' and RS, 'Ross Sea') (Roeder *et al.* 2001). Modern populations inhabiting Victoria Land in the Ross Sea present a mixture of both lineages, with frequencies of the Ross Sea 'RS' lineage increasing with increasing latitudes (Ritchie *et al.* 2004, Younger *et al.* 2015). Thus, different colonization patterns may have occurred at differing geographical and temporal scales (Emslie *et al.* 2007). In this region, penguin occupation occurred after ice sheets retreated following the Last Glacial Maximum (i.e. when the open sea and the ice-free land became available to the Adélie penguin for foraging and breeding) (Ainley 2002, Emslie *et al.* 2007). Deposits of subfossil bones and eggshells reveal that the species inhabited central Victoria Land since 8000 bp, but at different temporal and geographical scales (Emslie *et al.* 2007). During this period, gene frequencies have changed at the population level (Shepherd *et al.* 2005). Shepherd *et al.* (2005) concluded that micro-evolutionary changes among modern Adélie penguin populations may have been promoted by altered dispersal as a consequence of stochastic events creating physical barriers, such as mega-icebergs.

Genetic structure may be highly variable across many seabird species, and particularly among penguins living in different geographical areas (Clucas *et al.* 2014, Gorman *et al.* 2017). According to more recent studies, however, gene flow among pygoscelid colonies should be low and population genetic structure should be expected (for strongly philopatric species, see Ainley 2002, Vianna *et al.* 2017). On the other hand, a lack of genetic structure has been described for penguin species living at more northern latitudes (Clucas *et al.* 2014, Gorman *et al.* 2017, Cole *et al.* 2019). Similarly, Roeder *et al.* (2001), using six polymorphic microsatellite loci, detected no genetic differentiation among colonies of Adélie penguins throughout the Antarctic continent. Those findings have been confirmed both by Clucas *et al.* (2014) and by Gorman *et al.* (2017), who focused on colonies of the western Antarctic Peninsula.

Central place foragers such as penguins breed in colonies, and their distribution in the Antarctic varies according to density-dependent regulation, affected by the quality of the marine and terrestrial habitat (i.e. the presence of polynya and submarine canyons and the availability of terrestrial ice-free habitat) (Southwell & Emmerson 2020, Santora *et al.* 2020). Among Antarctic penguins, the Adélie penguin is a highly abundant sea ice-obligate species and as such it is considered as a key indicator species for both land and ocean environments (cf. Ainley 2002). The Adélie penguin winters in the pack ice surrounding the continent, subject to large-scale ice movement (e.g. Ballard *et al.* 2010), and, during spring, individuals reach ice- and snow-free sites along the coast
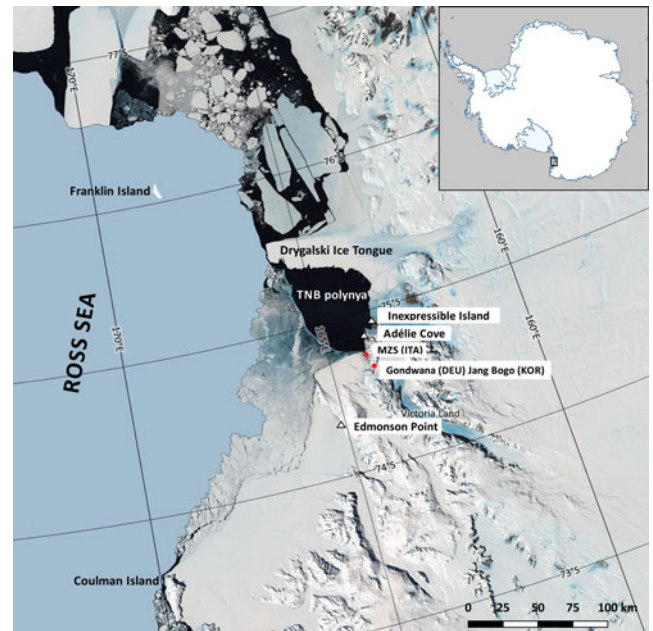
Fig. 1. Map of the Victoria Land coast and location of the three colonies. Location of the Italian research station MZS is also shown. Landsat image from Qantarctica3 (https://www.npolar.no/quantarctica), LIMA Landsat image mosaic of Antarctica (15/240 m) USGS/Remote Sensing of Environment, 2008. MZS = Mario Zucchelli Station; TNB = Terra Nova Bay.

to occupy breeding colonies (Ainley 2002, Lynch & LaRue 2014). During the breeding season, from October to February, 31 discrete colonies occur along the Victoria Land shoreline and together contribute ~33% of the total global population (Lynch & LaRue 2014). The colonies occur in clusters, associated with polynyas, with smaller ones neighbouring a large colony (Ainley 2002, Santora *et al.* 2020).

The Adélie penguin is philopatric (i.e. it shows high nest and colony fidelity, as well as minimal juvenile dispersal) (Ainley 2002, Dugger *et al.* 2010), and, as a consequence, a low level of gene flow is expected between populations (Nei 1987). Nevertheless, the dynamics of contemporary populations are pivotal in understanding population fluctuations of this species in a changing environment (LaRue *et al.* 2013, Dugger *et al.* 2014). How colonies occurring in a common geographical area relate to each other, or with much more distant colonies, is still little understood, although dispersal can affect population dynamics at the regional scale. Neighbouring colonies, not isolated but spatially separated, may interact as a meta-population (i.e. an assemblage of spatially delimited local populations that are coupled by some degree of migration) (Hanski & Gaggiotti 2004). Molecular genetic markers, particularly microsatellite loci, have increased our understanding of the

Table I. Ecological features of the three colonies located in central Victoria Land, Ross Sea, Antarctica. The linear distance to Terra Nova Bay polynya and range of fast-ice extension were measured using EOSDIS Worldview (https://worldview.earthdata.nasa.gov) to the best image available in late October, mid-December and mid-January from 2000 to 2018. The approximate size of colony area was measured with Google Earth Pro on images 23 February 2010, 27 November 2011 and 2 December 2011 for Edmonson Point, Adélie Cove and Inexpressible Island, respectively.

	Breeding population size (n occupied nests)	Colony area (km²)	Skua:penguin nests ratio	Average range of fast-ice extension during breeding period (max–min, km)	Linear distance to polynya (km)
Edmonson Point	2704[a]	0.03	1:28[b]	34–10	44
Adélie Cove	13,408[c]	0.06	1:654[b]	2–0	0
Inexpressible Island	36,117[c]	0.25	1:1250[b]	1–0	0

[a]Data from ground census of 29 November 2017, this study.
[b]Data from ground census (December 2017: S. Olmastroni, unpublished data 2017).
[c]Data for December 2013 from http://www.penguinmap.com/mapppd.

demographic history and phylogenetic relationships of penguin species (Roeder *et al.* 2001, Gorman *et al.* 2017, Vianna *et al.* 2017).

Here, using microsatellite loci previously isolated through next-generation sequencing (Roeder *et al.* 2001, Shepherd *et al.* 2005, Gorman *et al.* 2017), we assessed the small-scale population genetic structure of Adélie penguins from three adjacent colonies situated along the central Victoria Land coast. Inter-colony dynamics through population genetics have never been analysed among these colonies. We discuss our results in the context of meta-population dynamics (Hanski & Gaggiotti 2004, Bicknell *et al.* 2014) considering colony size, habitat quality and possible environmental forcing and evolutionary biology.

Material and methods

Study area

Our study took place from 10 November 2017 to 7 February 2018 in a cluster of three colonies occurring between Wood Bay and Terra Nova Bay (TNB), in a 75 km stretch of coast along central Victoria Land, Ross Sea (Antarctica) (Fig. 1): Inexpressible Island (74°54'S, 163°39'E), Adélie Cove (74°46'S, 164°00'E) and Edmonson Point (74°20'S, 165°08'E). The cluster is isolated, with the closest colonies ~200 km from one another (south-east: Franklin Island; north: Coulman Island) (Fig. 1).

Marine habitat

The TNB area has a unique marine fauna and shows high diversity at both the species and community levels (Povero *et al.* 2004). Oceanographic characteristics are dominated by the presence of the 'latent heat' TNB polynya, where strong and persistent katabatic winds drive newly formed sea ice offshore. Here, high primary production and strong benthic-pelagic coupling support species- and biomass-rich benthic and water-column communities (Davis *et al.* 2017). The Drygalski Ice Tongue plays an important role as a regulator of the size of the TNB polynya (Mezgec *et al.* 2017). Accordingly, the TNB area is a hotspot and a nursery area for the pelagic silverfish (*Pleuragramma antarctica*), a key species in the coastal food web, also supporting abundant crystal krill (*Euphausia crystallorophias*) stocks (Davis *et al.* 2017). Accordingly, the TNB area contains several colonies of Adélie penguin, Emperor penguin (*Aptenodytes forsteri*), south polar skua (*Stercorarius maccormicki*) and other flying seabirds (Harris *et al.* 2015) and marine mammals (e.g. seals and cetaceans: Lauriano *et al.* 2011, LaRue *et al.* 2019).

Colony habitat

The Adélie penguin colony at Inexpressible Island occupies ice-free ground on the eastern shore, where terrain is relatively flat, with a ridge of ~110 m above sea level (a.s.l.) elevation along the western flank. The area is strongly affected by katabatic winds flowing down a glacial valley towards the sea. The breeding population size in 2013 was 36,117 pairs (P.O.B. Lyver, personal observation 2013, Adélie census data, unpublished dataset, www.penguinmap.com) (Table I).

To the north, 17 km away, is the colony at Adélie Cove, a small 70 m-deep bay. The colony is located on a steep slope (80 m a.s.l.) and nests are distributed on ridges. The area is affected by katabatic winds. The breeding populations size in 2013 was 13,408 pairs (P.O.B. Lyver, personal observation 2013, Adélie census data, unpublished dataset, www.penguinmap.com) (Table I). Further north, 75 km from Inexpressible Island, is the colony at Edmonson Point, in a small ice-free area (1.79 km²) in Wood Bay. The colony consists of two main groups of nests located 600 m apart, the larger on a beach and the smaller on a volcanic terrace with a mild slope (25 m a.s.l.). The area is generally not affected by katabatic winds, and thus possesses several kilometres of fast ice that remains in place until late February. The breeding population size in 2017 was 2704 pairs (S. Olmastroni, unpublished data 2017). Terrestrial predators (i.e. south polar skuas) occur at each colony

Table II. Characterization of microsatellite loci of Adélie penguins used in our work.

Locus name	Repeated sequence	$T_{annealing}$	Size range (bp)	First reference
AM3	$A_xN_y(TA)_z$	55°C	171–176	Roeder *et al.* (2001)
AM12	$(CA)_x$	55°C	140–154	Roeder *et al.* (2001)
AM13	$A_xN_y(GT)_z(GC)_k(GT)_j$	50°C	110–156	Roeder *et al.* (2001)
RM3	$(CA)_x$	56°C	216–247	Roeder *et al.* (2001)
RM6	$(CA)_x$	57°C	168–180	Roeder *et al.* (2001)
XVCII	$(CA)_x$	58°C	75–101	Shepherd *et al.* (2005)
TP500	$(CA)_x$	62°C	106–126	Roeder *et al.* (2001)

(Table I), as do marine predators (i.e. Leopard seals *Hydrurga leptonyx*) at adjacent beaches. Killer whales (*Orcinus orca*, ecotype B) have been observed travelling along the fast-ice edge and shoreline among the Wood Bay and TNB area (Lauriano *et al.* 2011). A previous study showed that the penguins fed little inside the TNB polynya area, but rather used it to reach the pack ice that rings the polynya, rather than to walking (Olmastroni *et al.* 2020).

Data collection

At the beginning of the breeding season (early November), a total of 50 occupied nests were selected in each colony for carrying out different research activities in the framework of the PenguinERA project (PNRA2016 AZ1.11) and for the long-term monitoring of the Adélie penguins in the TNB area (cf. Olmastroni *et al.* 2020). An Adélie penguin colony is composed of numerous separated subgroups of different sizes and shapes (Ainley 2002); therefore, we attempted to balance sampling spatially. In particular, both peripheral and central breeders (see Tenaza 1971) in different subgroups within each colony were selected to limit differences in nest characteristics (e.g. nesting success and colony growth) and to maximize distance among individuals (i.e. to avoid inbreeding and relatedness) (cf. Cristofari *et al.* 2015). As our study was carried out throughout the breeding period, no central nests located beyond three nests from the colony edge were sampled in order to limit disturbance to the other breeders. Up to six feathers per individual were plucked from the chest area. To reduce stress of capturing and handling, adult penguins were gently lifted from their nests and covered with a hood, while eggs and/or chicks were protected and kept warm during handling. Penguins were marked with passive transponders (TIRIS™ Texas Instruments Registration and Identification System) and externally with a temporary dye to avoid recapturing the same individual. We released each penguin in front of its nest after a 10 min maximum holding time and observed individuals until they returned to their regular breeding activity. Feather samples for this study were collected from 30 adult penguins per colony within the 50 relevant

previously marked nests and stored in sealed plastic bags at -20°C until genetic analysis was conducted.

DNA extraction, amplification and sequencing

Two feathers per individual were used for DNA extraction. The total genome DNA was extracted successfully from 26–32 individuals per colony (Edmonson Point, $n = 28$; Adélie Cove, $n = 32$; Inexpressible Island, $n = 26$) through the QIAamp DNA Micro Kit (©Qiagen 2013–18) following the Isolation from Tissues Protocol. Analyses were then normalized for the smallest sample. The DNA content was quantified with a NanoDrop ND1000UV (©NanoDrop Technologies) then stored at 4°C. Polymerase chain reactions (PCRs) were carried out for seven microsatellite loci (Table II) widely used in previous studies, so as to allow comparison with other works on the same species (Roeder *et al.* 2001, Shepherd *et al.* 2005, Gorman *et al.* 2017). The PCRs were run in a total volume of 25 µl containing at least 2 µl of DNA at 20 ng/µl, 0.4 µM of each primer, 200 µM of each dNTP, 1.5 mM of $MgCl_2$, 1× reaction buffer and 0.125 µl of GoTaq Flexi DNA Polymerase (5 units/µl) (©Promega).

After the electrophoretic run on 1.5% agarose gel, a solution containing 1 µl of PCR product was prepared for each amplified DNA diluted with sterile distilled water in an amount proportional to the intensity of the band, containing 13.5 µl of deionized formamide (denaturing agent; ©AppliChem) and 0.3 µl of a size standard (ROX 500 size standard; ©Applied Biosystems). Then, we proceeded with a denaturation step of 10 min at 94°C. Samples were run using an automatic multicapillary sequencer (ABI Prism 373, ©Applied Biosystems) at the Department of Life Sciences of the University of Siena. The software *Geneious 6.0.3* (Kearse *et al.* 2012) was used to establish the precise assignment of amplified allelic sizes. The obtained results were displayed on a two-dimensional graph, with the abscissa corresponding to the length (bp) and the ordinate to the peak height (fluorescence unit). The height of the peaks, the intensity of which being proportional to the amount amplified, has the same meaning as the intensity of the electrophoresis band on agarose gel. The allelic range was calculated for
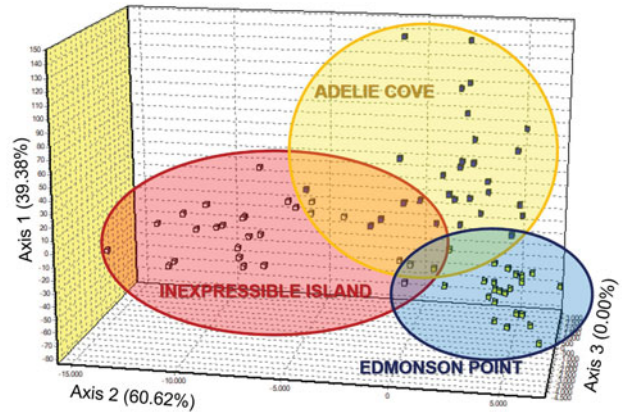
Table III. Polymorphic informative content (PIC) and allelic richness for each locus and for each colony, corrected for population size.

| Locus	PIC	Allelic richness corrected per sample size			
		Edmonson Point	Adélie Cove	Inexpressible Island	Total
AM3	0.313	1.929	2.000	4.000	3.763
AM12	0.487	2.000	3.967	5.000	4.550
AM13	0.637	2.929	4.000	7.000	6.066
RM3	0.492	2.000	5.592	4.000	4.655
RM6	0.416	2.000	2.967	4.000	3.069
XVCII	0.796	3.000	9.960	8.000	10.034

Fig. 3. Tridimensional factorial correspondence analysis, including all of the sampled individuals (yellow = Adélie Cove; red = Inexpressible Island; blue = Edmonson Point). Axes show the variables explaining the variability of the genetic diversity in our dataset.

each locus on the basis of the maximum and minimum lengths found for the analysed samples.

Statistical analyses of the microsatellite data

We assessed whether null alleles (i.e. non-amplified), allelic dropouts and errors due to stutter peaks occurred, and the polymorphic informative content (PIC) was also assessed using standard methods (see Supplementary Material). We computed allelic richness (i.e. the number of alleles per locus corrected per sample size), allelic frequencies and private alleles (i.e. those occurring only in one colony and not detected in the others), Nei's genetic diversity index and the values of the F_{ST} parameter according to Wright's F-statistics (i.e. the measure of genetic differentiation among colonies). Deviations from the Hardy-Weinberg equilibrium (HWE) and from linkage disequilibrium (LE) were also calculated for each locus and for the total loci per colony. The significance level for the HWE and LE was estimated using a Bonferroni correction. We estimated gene flow, expressed as the number of migrants among colonies per generation, corrected for population size (i.e. the average number of migrants successfully entering each population per generation). To conclude, we performed a cluster analysis on the microsatellite (short tandem repeat, STR) genotypic profile of each individual using the Bayesian

method (Supplementary Material). The probability that each individual belonged to each of the clusters defined by the program was calculated (Supplementary Material).

Results

Six of the seven microsatellite loci chosen for this study were successfully amplified in all samples. The high number of non-specific bands (i.e. 4–6) obtained in the electrophoretic run of the locus TP500, despite all precautions applied (i.e. addition of dimethyl sulfoxide in PCR, alteration of the concentration of $MgCl_2$, increases in the annealing temperature; see Roeder *et al.* 2001), made the locus almost useless. Several samples sequenced for this locus (TP500), considering its length based on published data (Roeder *et al.* 2001, Shepherd *et al.* 2005), were monomorphic in the three study
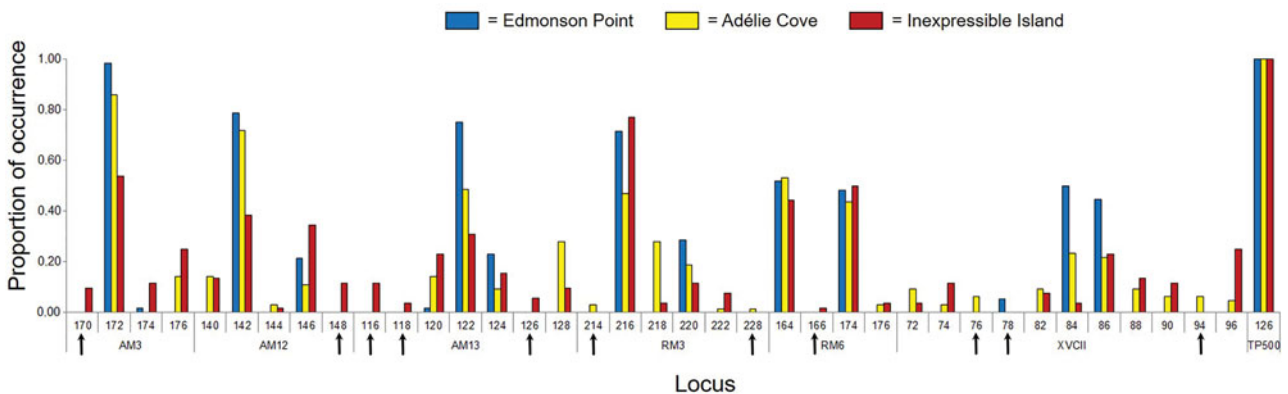

Fig. 2. Allelic frequencies for each locus for all studied colonies: arrows on the x-axis show private alleles.

Table IV. Hardy-Weinberg equilibrium multilocus test and tests for deficiency and excess of heterozygotes.

Colony	Multilocus test (P)	Heterozygote deficiency (P)	Heterozygote excess (P)
Edmonson Point	0.004	0.004	<< 0.001
Adélie Cove	<< 0.001	<< 0.001	<< 0.001
Inexpressible Island	<< 0.001	<< 0.001	<< 0.001

Table V. Results of the analysis of molecular variance between colonies (F_{ST}) and Nei's diversity indices for genetic diversity (average gene diversity ± standard deviation).

Colony	Edmonson Point	Adélie Cove	Inexpressible Island	Nei's diversity index
Edmonson Point	-			0.322 ± 0.199
Adélie Cove	0.074 ($P < 0.05$)	-		0.492 ± 0.283
Inexpressible Island	0.148 ($P < 0.05$)	0.071 ($P < 0.05$)	-	0.667 ± 0.220
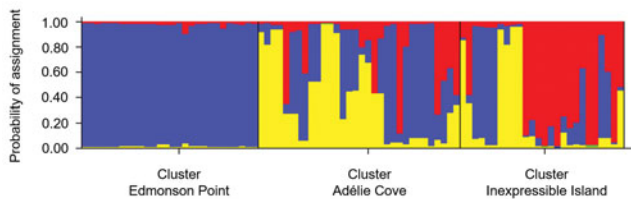
Fig. 4. Probabilities of assignment of Adélie penguin individuals to putative population clusters following the Bayesian analysis of population structure for six microsatellite loci per individual. Each individual penguin is represented by vertical lines and different colours represent different clusters (blue = Edmonson Point; yellow = Adélie Cove; red = Inexpressible Island). The structure plot was obtained without prior location information.

colonies (PIC = 0). Therefore, the TP500 locus was excluded from further analysis.

Analyses carried out with *MicroChecker* did not show any evidence of stutter bands, nor allelic dropout. Amplified loci showed a medium to high PIC values (Table III). The majority of the unique alleles (i.e. characteristic of a population) were found for the largest colony (i.e. Inexpressible Island; $n = 6$). Consistently, the lowest number of private alleles ($n = 1$) was observed at Edmonson Point (Fig. 2).

The allelic richness corrected for the population size reached a maximum in all comparisons for the XVCII locus (up to 9.9). At the population level, the lowest values were observed for Edmonson Point (range: 1.9–3.0) and the highest values were observed for Inexpressible Island (range: 4.0–8.0), apart from the

locus XVCII in Adélie Cove (allelic richness: 9.9) (Table III). The three-dimensional factorial correspondence analysis showed the presence of three well-defined clusters (permutation test of θ using 1000 re-samplings = 0.095–0.224, $P < 0.01$ in all pairwise comparisons) (Fig. 3). Our study colonies showed no significant deviation from the HWE (all $P < 0.005$) (Table IV). Pairwise comparisons of all loci assessed by a drop-down menu of the software *Genepop* showed no LD for population genetics analysis. Therefore, the association of alleles in different loci in the investigated colonies was random. The F_{ST} values (Table V) were significant (all $P < 0.05$), with the highest value found in the comparison between the largest and the smallest colonies (i.e. Inexpressible Island and Edmonson Point, respectively) (Table V). Nei's diversity index confirmed that the highest value of genetic diversity was found for the Inexpressible Island colony (0.67 ± 0.22) (Table V).

Gene flow between colonies is limited to four emigrating individuals every three generations (number of emigrating individuals corrected for population size = 1.33 per generation over the sampled population), thus showing isolation among populations. The Bayesian analysis of the genotyping of the three colonies confirmed a subdivision into three clusters (Fig. 4); ΔK (i.e. an *ad hoc* quantity based on the second-order change rate of the likelihood function with respect to K) was the highest for $K = 3$.

Discussion

In our work, microsatellite data revealed limited dispersal in Adélie penguins among an isolated cluster of three adjacent colonies in Victoria Land. A significant level of population genetic structure was detected, greater than what has been observed among other colonies of this species using polymorphic markers (Roeder *et al.* 2001, Gorman *et al.* 2017). The F_{ST} values, which represent the degree of divergence between subpopulations (i.e. colonies; Nei 1987), were greater than those observed among populations of Adélie and other penguin species whose nesting sites are separated by even larger geographical distances (Younger *et al.* 2015, Gorman *et al.* 2017). The power of our findings may have been higher with a larger set of markers and a greater sample size (Gorman *et al.* 2017). Our results are comparable with mitochondrial DNA data previously tested in our study colonies, which showed the lowest diversity levels of the Ross Sea and the highest relative abundance of the 'A' lineage with respect to the 'RS' lineage (Ritchie *et al.* 2004, Younger *et al.* 2015). Moreover, our results are comparable with those of Roeder *et al.* (2001), who similarly used seven microsatellite markers but detected no genetic structure.

Allelic richness and genetic diversity (i.e. Nei's diversity index) were highest in the large Inexpressible Island (including ~36,110 nests) colony and lowest in the small Edmonson Point colony (including ~2700 nests). Distributions of private alleles followed the same trend. We observed high homogeneity within each colony, with some overlap. Most variability was observed among colonies, which may represent three divergent demes. Accordingly, gene flow was the lowest between the most distant colonies (i.e. Edmonson Point and Inexpressible Island) and was highest between the Inexpressible Island colony and the intermediate one of Adélie Cove.

Although we evaluated a lower number of colonies than previous studies on the population genetics of the Adélie penguin, such studies did not show any population genetic structure among modern colonies, suggesting a higher level of dispersal (Roeder *et al.* 2001, Gorman *et al.* 2017). Geographical, environmental (i.e. including historical trophic and climatic) and behavioural constraints may influence dispersal distances, sometimes increasing individual movements among breeding colonies (Roeder *et al.* 2001, Clucas *et al.* 2014, Gorman *et al.* 2017). Lack of genetic structure in previously studied populations of the Adélie penguin has been related to the high number of emigrant individuals per generation among colonies corrected for population size ($Nm_s = 6$–9) (Roeder *et al.* 2001, Gorman *et al.* 2017), possibly triggered by local climate change effects. Accordingly, climatic change appears to force penguins to move towards areas that better fulfil their ecological requirements in terms of habitat suitability and food availability during chick rearing (LaRue *et al.* 2013, Gorman *et al.* 2017). In the Antarctic Peninsula, where Gorman *et al.* (2017) carried out their study, large colonies of the only 'A' lineage occur and climate-driven alterations may have increased dispersal movements, resulting in a panmictic population (Clucas *et al.* 2014, Gorman *et al.* 2017). Conversely, where climatic conditions are more stable or cooling (e.g. in our study areas, or at the southern-most latitudes of the Ross Sea), philopatry and dispersal may be more predictable (Gorman *et al.* 2017). In other words, where climatic and environmental changes are less remarkable or conditions are more stable (e.g. in the Ross Sea), Adélie penguins appear to show higher philopatry (see also Dugger *et al.* 2014). This would in turn limit the emigration rate among colonies in the Ross Sea, resulting in an evident genetic structure among colonies (i.e. high inter-colony heterogeneity), which has not been observed in warming areas in Antarctica (Clucas *et al.* 2014, Gorman *et al.* 2017).

The Adélie penguin has coped with climate variability of the Southern Ocean over geological time. Population fluctuations in different areas have been the consequence of periods of abandonment during cooling ages (e.g. during the Last Glacial Maximum) followed by recolonization when new terrestrial breeding sites and open water became available (i.e. when ice sheets retreat following ice ages) (Younger *et al.* 2015). Radiocarbon dating from ornithogenic soils sampled at both abandoned and modern penguin colonies has shown Adélie penguin occupation histories and temporal fluctuations in the Ross Sea region over the last 45,000 years (Emslie *et al.* 2007). Accordingly, phylogenetic studies have found evidence that both monophyletic lineages of Adélie penguins (Ritchie *et al.* 2004, Younger *et al.* 2015) occur in Victoria Land, which may reflect the presence of a well-supported population genetic structure.

The opening of the western Ross Sea (Victoria Land coast) allowed the southern TNB area to be the first area to be reoccupied by Adélie penguins by ~8.2–7.7 ka (Mezgec *et al.* 2017), followed by two subsequent periods of abandonment due to unfavourable breeding conditions. Thus, following ice ages and interglacial periods, more recent reoccupation of central Victoria Land started *ca*. 5000–4000 BP. Edmonson Point was occupied *ca*. 2000–1100 BP (Emslie *et al.* 2007). The genetic structure and differences observed in our and previous studies (e.g. Ritchie *et al.* 2004) may reflect the population history of the Adélie penguin in the study areas following ice advances and retreats and subsequent colonization and immigration patterns.

Shepherd *et al.* (2005) compared the radiocarbon of ancient remains in ornithogenic soils with samples from modern colonies (i.e. Inexpressible Island). They highlighted that genetic variability in Adélie penguin populations of Victoria Land was probably affected by mega-icebergs. Icebergs break off from the retreating West Antarctic Ice Sheet and Ross Ice Shelf, periodically changing environmental conditions, which trigger the mixing of the populations through heightened emigration/immigration. Calving events, and the giant icebergs generated, are indeed responsible for the alteration of sea-ice circulation, polynya openings and the marine food web elsewhere in coastal Antarctica at different times (Michel *et al.* 2019). Such events may cause penguin colony breeding failure, as adults are wholly prevented from colony access when icebergs and extensive sea ice may become physical barriers to reach foraging areas or for returning to the nest in time to feed chicks (Dugger *et al.* 2014, Ropert-Coudert *et al.* 2018). In central Victoria Land, the Drygalski Ice Tongue (Fig. 1) is an important regulator of the size of the TNB polynya (Davis *et al.* 2017). Thus, major and minor ice-calving events may have altered sea-ice cover and shaped the marine environment and fauna near the TNB colonies (Mezgec *et al.* 2017).

The distribution and growth of Adélie penguin colonies are regulated by density-dependent factors, in addition to

the quality of marine and terrestrial habitats (Ainley 2002, Lyver *et al.* 2014, Southwell & Emmerson 2020). According to the source-sink theory (Hanski & Gaggiotti 2004), Inexpressible Island in particular as well as Adélie Cove may be 'high-quality' habitats and act as source populations, whereas Edmonson Point may represent a sink. A small population - despite available breeding ground - may be the result of the occupation of a low-quality patch. Small colonies that are more isolated (e.g. by fast ice, as in our case) have been described as being at a higher risk of extinction compared to large colonies (Bicknell *et al.* 2014, Southwell & Emmerson 2020). Penguin access to Edmonson Point is limited by extensive fast ice throughout the breeding season. Edmonson Point also has the highest numbers of terrestrial predators (Table I). Skua predation is the major cause of egg and chick loss, accounting for up to 40% during the breeding season (S. Olmastroni, unpublished data 1996–2018). However, breeding success, diet, foraging range and adult survival rate for the Edmonson Point population are similar to those of other colonies (Ballerini *et al.* 2009, Olmastroni *et al.* 2020) and other parts of the Antarctic (Jenouvrier *et al.* 2006). Immature pre-breeders are important to population demography (Bicknell *et al.* 2014), although Ballerini *et al.* (2009) reported a low recruitment of birds marked as chicks (25%) for this colony.

Thus, the Edmonson Point colony, characterized by low recruitment rates as well as risky and more 'stressful' habitats (higher numbers of terrestrial predators and extensive fast ice) (Table I), may be considered a low-quality patch. Indeed, the colony seems at risk of extinction and is only able to maintain a stable population size by being sustained by immigration from other colonies (Ballerini *et al.* 2015). Our present results of low genetic variability within this colony do not support this hypothesis. Quantification of the penguin immigration rate in southern Ross Sea colonies highlighted a rate of 1% population dispersal among neighbouring colonies, except for under unusual circumstances. Dugger *et al.* (2014) described how movements of breeding adults, survival and dispersal among clustered colonies may be altered by giant icebergs that separate colonies and also trap extensive sea ice, causing stressful conditions for several seasons. Under relatively stable environmental conditions, the degree of philopatry of the Adélie penguin, an adequate number of pre-breeders who survive to return to breed and a small immigration rate from other colonies may explain the maintenance of the Edmonson Point population, the low gene flux among colonies and the high genetic homogeneity within this colony (Fig. 4).

Our findings on genetic homogeneity within populations and an evident genetic structure amongst colonies contribute to insights into the population dynamics of a local cluster of Adélie penguin colonies. In fact, Inexpressible Island, Adélie Cove and Edmonson Point appear to be somewhat independent subpopulations maintained by a minimal dispersal mechanism and may thus interact as a meta-population. Future research would benefit from a more comprehensive set of markers and a larger sample size to compare results with other studies (e.g. Ritchie *et al.* 2004, Clucas *et al.* 2014, Gorman *et al.* 2017). Moreover, further studies of the population genetic structure and dispersal are required to investigate the population dynamics and genetic structure of Adélie penguin populations in the TNB area. Increasing the study of penguin movements amongst colonies in the Ross Sea region over the long term would provide vital knowledge for the management and conservation of this long-lived seabird species.

Supplementary material

A supplemental methods section will be found at https://doi.org/10.1017/S0954102021000067.

Author contributions

EM participated in conceiving this study, carried out genetic and relevant statistical analyses and drafted the manuscript. CB carried out genetic and relevant statistical analyses. AC supervised the molecular analysis. LB participated in planning the fieldwork and carried out the fieldwork. NF participated in planning the fieldwork, carried out the fieldwork and participated in writing. FF participated in conceiving this study and writing. SO conceived and designed this study, coordinated the research, planned and coordinated the

fieldwork, carried out the fieldwork, participated in interpreting the data and drafted the manuscript. All authors read and approved the final manuscript.

Ethical approval

All sampling followed SCAR's Code of Conduct for the Use of Animals for Scientific Purposes in Antarctica (https://www.scar.org/policy/scar-codes-of-conduct) and under permission released from PNRA ('Programma Nazionale di Ricerche in Antartide').

Details of data deposit

The datasets generated and/or analysed during the current study are stored at University of Siena and Museo Nazionale dell'Antartide in Siena and are available from the corresponding author upon reasonable request.

References

AINLEY, D.G. 2002. *The Adélie penguin. Bellwether of climate change.* New York, NY: Columbia University Press, 416 pp.

BALLARD, G., TONIOLO, V., AINLEY, D.G., PARKINSON, C.L., ARRIGO, K.R. & TRATHAN, P.N. 2010. Responding to climate change: Adélie penguins confront astronomical and ocean boundaries. *Ecology*, **91**, 2056–2069.

BALLERINI, T., TAVECCHIA, G., OLMASTRONI, S., PEZZO, F. & FOCARDI, S. 2009. Nonlinear effects of winter sea ice on the survival probabilities of Adélie penguins. *Oecologia*, **161**, 253–265.

BALLERINI, T., TAVECCHIA, G., PEZZO, F., JENOUVRIER, S. & OLMASTRONI, S. 2015. Predicting responses of the Adélie penguin population of Edmonson Point to future sea ice changes in the Ross Sea. *Frontiers in Ecology and Evolution*, **3**, 8.

BICKNELL, A.W., KNIGHT, M.E., BILTON, D.T., CAMPBELL, M., REID, J.B., NEWTON, J. & VOTIER, S.C. 2014. Intercolony movement of pre-breeding seabirds over oceanic scales: implications of cryptic age-classes for conservation and metapopulation dynamics. *Diversity and Distributions*, **20**, 1–9.

CLUCAS, G.V., DUNN, M.J., DYKE, G., EMSLIE, S.D., LEVY, H., NAVEEN, R., *et al.* 2014. A reversal of fortunes: climate change 'winners' and 'losers' in Antarctic Peninsula penguins. *Scientific Reports*, **4**, 10.1038/srep05024.

COLE, T.L., DUTOIT, L., DUSSEX, N., HART, T., ALEXANDER, A., YOUNGER, J.L., *et al.* 2019. Receding ice drove parallel expansions in Southern Ocean penguins. *Proceedings of the National Academy of Sciences of the United States of America*, **116**, 26690–26696.

COULSON, J.C. 2016. A review of philopatry in seabirds and comparisons with other waterbird species. *Waterbirds*, **39**, 229–240.

CRISTOFARI, R., TRUCCHI, E., WHITTINGTON, J.D., VIGETTA, S., GACHOT-NEVEU, H., STENSETH, N.C., *et al.* 2015. Spatial heterogeneity as a genetic mixing mechanism in highly philopatric colonial seabirds. *PLoS ONE*, **10**, 10.1371/journal.pone.0117981.

DAVIS, L.B., HOFMANN, E.E., KLINCK, J.M., PIÑONES, A. & DINNIMAN, M.S. 2017. Distributions of krill and Antarctic silverfish and

correlations with environmental variables in the western Ross Sea, Antarctica. *Marine Ecology Progress Series*, **584**, 45–65.

DUGGER, K.M., AINLEY, D.G., LYVER, P.O.B., BARTON, K. & BALLARD, G. 2010. Survival differences and the effect of environmental instability on breeding dispersal in an Adélie penguin meta-population. *Proceedings of the National Academy of Sciences of the United States of America*, **107**, 12375–12380.

DUGGER, K.M., BALLARD, G., AINLEY, D.G., LYVER, P.O.B. & SCHINE, C. 2014. Adélie penguins coping with environmental change: results from a natural experiment at the edge of their breeding range. *Frontiers in Ecology and Evolution*, **2**, 68.

EMSLIE, S.D., COATS, L. & LICHT, K. 2007. A 45,000 yr record of Adélie penguins and climate change in the Ross Sea, Antarctica. *Geology*, **35**, 61–64.

GORMAN, K.B., TALBOT, S.L., SONSTHAGEN, S.A., SAGE, G.K., GRAVELY, M.C., FRASER, W.R. & WILLIAMS, T.D. 2017. Population genetic and gene flow of Adélie penguins (*Pygoscelis adeliae*) breeding throughout the Western Antarctic Peninsula. *Antarctic Science*, **29**, 499–510.

HANSKI, I. & GAGGIOTTI O.E. (eds.) 2004. *Ecology, genetics and evolution of metapopulations.* Cambridge, MA: Academic Press, 696 pp.

HARRIS, C.M., LORENZ, K., FISHPOOL, L.D.C., LASCELLES, B., COOPER, J., CORIA, N.R., *et al.* 2015. *Important bird areas in Antarctica 2015.* Cambridge: BirdLife International and Environmental Research and Assessment Ltd, 45 pp.

JENOUVRIER, S., BARBRAUD, C. & WEIMERSKIRCH, H. 2006. Sea ice affects the population dynamics of Adélie penguins in Terre Adélie. *Polar Biology*, **29**, 413–423.

KEARSE, M., MOIR, R., WILSON, A., STONES-HAVAS, S., CHEUNG, M., STURROCK, S., *et al.* 2012. *Geneious Basic*: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics*, **28**, 1647–1649.

LARUE, M.A., AINLEY, D.G., SWANSON, M., DUGGER, K.M., LYVER, P.O., BARTON, K. & BALLARD, G. 2013. Climate change winners: receding ice fields facilitate colony expansion and altered dynamics in an Adélie penguin metapopulation. *PLoS ONE*, **8**, e60568.

LARUE, M.A., SALAS, L., NUR, N., AINLEY, D.G., STAMMERJOHN, S., BARRINGTON, L., *et al.* 2019. Physical and ecological factors explain the distribution of Ross Sea Weddell seals during the breeding season. *Marine Ecology Progress Series*, **612**, 193–208.

LAURIANO, G., FORTUNA, C.M. & VACCHI, M. 2011. Occurrence of killer whales (*Orcinus orca*) and other cetaceans in Terra Nova Bay, Ross Sea, Antarctica. *Antarctic Science*, **23**, 139–143.

LYNCH, H.J. & LARUE, M.A. 2014. First global census of the Adélie penguin. *The Auk*, **131**, 457–466.

LYVER, P.O.B., BARRON, M., BARTON, K.J., AINLEY, D.G., POLLARD, A., GORDON, S., *et al.* 2014. Trends in the breeding population of Adélie penguins in the Ross Sea, 1981–2012: a coincidence of climate and resource extraction effects. *PLoS ONE*, **9**, e91188.

MEZGEC, K., STENNI, B., CROSTA, X., MASSON-DELMOTTE, V., BARONI, C., BRAIDA, M., *et al.* 2017. Holocene sea ice variability driven by wind and polynya efficiency in the Ross Sea. *Nature Communications*, **8**, 1334.

MICHEL, L.N., DANIS, B., DUBOIS, P., ELEAUME, M., FOURNIER, J., GALLUT, C., *et al.* 2019. Increased sea ice cover alters food web structure in East Antarctica. *Scientific Reports*, **9**, 8062.

MILOT, E., WEIMERSKIRCH, H. & BERNATCHEZ, L. 2008. The seabird paradox: dispersal, genetic structure and population dynamics in a highly mobile, but phylopatric albatross species. *Molecular Ecology*, **17**, 10.1111/j.1365-294X.2008.03700.x.

NEI, M. 1987. *Molecular evolutionary genetics.* New York, NY: Columbia University Press, 514 pp.

OLMASTRONI, S., FATTORINI, N., PEZZO, F. & FOCARDI, S. 2020. Gone fishing: Adélie penguin site-specific foraging tactics and breeding performance. *Antarctic Science*, **32**, 199–209.

POVERO, P., CASTELLANO, M., RUGGIERI, N., MONTICELLI, L.S., SAAGIOMO, V., MARIACHIARA CHIANTORE, M.C., *et al.* 2004. Water column features

and their relationship with sediments and benthic communities along the Victoria Land coast, Ross Sea, summer 2004. *Antarctic Science*, **18**, 10.1017/S0954102006000642.

RITCHIE, P.A., MILLAR, C.D., GIBB, G.C., BARONI, C. & LAMBERT, D.M. 2004. Ancient DNA enables timing of the Pleistocene origin and Holocene expansion of two Adélie penguin lineages in Antarctica. *Molecular Biology and Evolution*, **21**, 240–248.

ROEDER, A.D., MARSHALL, R.K., MITCHELSON, A.J., VISAGATHILAGAR, T., RITCHIE, P.A., LOVE, D.R., *et al.* 2001. Gene flow on the ice: genetic differentiation among Adélie penguin colonies around Antarctica. *Molecular Ecology*, **10**, 1645–1656.

ROPERT-COUDERT, Y., KATO, A., SHIOMI, K., BARBRAUD, C., ANGELIER, F., DELORD, K., *et al.* 2018. Two recent massive breeding failures in an Adélie penguin colony call for the creation of a marine protected area in D'Urville Sea/Mertz. *Frontiers in Marine Science*, **5**, 264.

SANTORA, J.A., LARUE, M.A. & AINLEY, D.G. 2020. Geographic structuring of Antarctic penguin populations. *Global Ecology and Biogeography*, **29**, 10.1111/geb.13144.

SHEPHERD, L.D., MILLAR, C.D., BALLARD, G., AINLEY, D.G., WILSON, P.R., HAYNES, G.D., *et al.* 2005. Microevolution and mega-icebergs in the Antarctic. *Proceedings of the National Academy of Sciences of the United States of America*, **102**, 16717–16722.

SOUTHWELL, C. & EMMERSON, L. 2020. Density dependence forces divergent population growth rates and alters occupancy patterns of a central place foraging Antarctic seabird. *Ecology & Evolution*, **10**, 2339–2351.

TENAZA, R. 1971. Behavior and nesting success relative to nest location in Adelie penguins (*Pygoscelis adeliae*). *The Condor* **73**, 81–92.

VIANNA, J.A., NOLL, D., DANTAS, G.P., PETRY, M.V., BARBOSA, A., GONZÁLEZ-ACUÑA, D., *et al.* 2017. Marked phylogeographic structure of Gentoo penguin reveals an ongoing diversification process along the Southern Ocean. *Molecular Phylogenetics and Evolution*, **107**, 486–498.

YOUNGER, J., EMMERSON, L., SOUTHWELL, C., LELLIOTT, P. & MILLER, K. 2015. Proliferation of East Antarctic Adélie penguins in response to historical deglaciation. *BMC Evolution Biology*, **15**, 236.