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Genomic analyses reveal trans-generational haul out site fidelity in leopard seals

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As top predators, leopard seals (*Hydrurga leptonyx*) have a key role in Southern Ocean ecosystems. For example, this species has driven the local collapse of Antarctic fur seals (*Arctocephalus gazella)* at Cape Shirreff, in the northern Antarctic Peninsula. However, little is known about leopard seal haul out site fidelity and social behavior. Here, we employ "genomic tagging" and relatedness analyses from a genome-wide single nucleotide polymorphism (SNP) dataset obtained from 88 leopard seal tissue samples to investigate patterns of seasonal haul out site fidelity and social structure at Cape Shirreff, a leopard seal hotspot during austral summers. Although many seals were observed only once, some females had remarkably high site fidelity returning to the same location across timeframes of up to eight years. Most leopard seals were unrelated, but we identified a trio of closely related females, including a mother-daughter pair, indicating that seasonal site fidelity can span generations. Interestingly, the mother leopard seal identified through our relatedness analyses is a foraging specialist that targets Antarctic fur seal pups; her diet changed very little over the past decade. Our findings suggest high individual variability in leopard seal behavior regarding site fidelity and social structure. Such flexibility may play a key role in this species' responses to environmental change.

Keywords Leopard seal, *Hydrurga leptonyx*, Relatedness, Kinship, Pinniped, ddRADseq, Seal

Decadal shifts in the behavior and diet of large marine predators can profoundly transform ecosystems through cascading predator–prey interactions[1–3]. The Southern Ocean supports one of the largest communities of seabirds and marine mammals in the world[4,5], and many of those populations are changing rapidly in the warming climate[6–9]. Our understanding of these community level changes depends upon long-term observations of predator populations. In particular, behavior and demographic data derived from known individuals are critical for contextualizing regional patterns (e.g.,[10–13]). However, acquiring long-term data for individuals is challenging for solitary, long-lived species that occur at low population densities[14]. Most assessments of social structure and kinship associations have focused on social species despite the fact that most carnivores are solitary (defined as individuals spending more than 50% of the time sleeping or foraging alone, and sexes meeting mainly for mating[15]). For solitary species, a combination of approaches is typically needed to acquire knowledge on habitat use, dispersal patterns and social systems[16].

Site fidelity is the tendency of individuals to return to a particular location over time and is commonly observed in gregarious pelagic sea birds and pinnipeds (seals, sea lions and walruses;[17]). Individual site fidelity associated with foraging, resting or molting (hereafter "site fidelity") shapes population dynamics by accelerating specialization and regulating intraspecific competition[18]. Most importantly, site fidelity allows individuals to gain familiarity with locally available resources and environmental conditions[19], which can increase reproductive success and lifespan (e.g., southern elephant seals, *Mirounga leonina*;[20]). Nevertheless, site fidelity can be variable in populations due to intrinsic factors, such as age and sex. Specifically, adults tend to have greater site fidelity, while younger animals are often more exploratory (e.g., southern elephant seals[21]; Weddell seals, *Leptonychotes*

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weddellii; [22]; northern gannets, *Morus bassanus*[23]). The two sexes can also have contrasting site fidelity patterns. For example, post-breeding sex-specific segregation is observed in the Pacific walrus (*Odobenus rosmarus divergens*); after winter breeding, females and juveniles migrate to the Chuckchi Sea while males remain in the Bering Sea[24]. In contrast, site fidelity can remain stable over many generations in some species (e.g., short-tailed albatross, *Phoebastria albatrus*[25]), making them highly susceptible to rapid changes in their environment. Notably, a mix of weak and strong individual site fidelity strategies can lead to long-term benefits at the population level. In northern elephant seals, *Mirounga angustirostris*, individuals with weak fidelity had higher energetic rewards in positive Pacific Decadal Oscillation years while individuals with strong fidelity had lower variability in mass across years, so both strategies were equally successful over a decade[26]. Understanding patterns of site fidelity is critical to revealing population dynamics that can inform species conservation strategies, particularly for long-lived vertebrates vulnerable to decline[27,28].

Social structure is directly linked to site fidelity. Social interactions are more common among individuals over time if they consistently occupy the same area. In highly social Galapagos sea lions (*Zalophus wollebaeki*), fine-scale site fidelity and space use can promote bonds between individuals, leading to community-level social structure[29,30]. There is also mounting evidence that sociality does not exclusively occur in group-living species[31]. Indirect, non-visual social signals (e.g., scent), for example, appear to determine the home ranges of many solitary mammals, including the puma (*Puma concolor*[32]), slender mongoose (*Galerella sanguinea*[33]), and brown bear (*Ursus arctos*[34]). In fact, some carnivore matrilineal societies observed today (e.g., lions, *Panthera leo*, and spotted hyenas, *Crocuta crocuta*) likely evolved from proximity tolerance among female kin and nepotistic transmission of critical resources from one generation to the next, leading to enhanced overall population fitness[35].

Leopard seals (*Hydrurga leptonyx*) are solitary Southern Ocean top predators, with a circumpolar distribution around the Antarctic pack ice[36], but the species also occurs north of the Antarctic Convergence, in Australia, New Zealand and Chile[37–40]. Breeding occurs in the austral spring with a peak of births reported between the months of September and December[38,41]. During the austral summers leopard seals are often observed foraging near large breeding aggregations of penguins and seals[42,43]. This is also the time of the year when leopard seals spend the greatest number of hours hauled out[44] and are accessible to researchers for studies involving tagging and capture. One of the locations visited by leopard seals in the summers is Cape Shirreff, located in Livingston Island (South Shetland Islands), northern Antarctic peninsula. At this location, no breeding or mating have been observed. Instead, leopard seals are seen hunting near shore or resting on beaches.

Leopard seals forage across a range of trophic levels from mesopredators (e.g., penguins and seals) to fish and krill[36,45]. They can rapidly restructure prey populations through top-down forcing[46,47]. For example, at Cape Shirreff leopard seals decimated South Shetland Antarctic fur seals in just over two decades by consuming nearly 70% of pups born annually since 2010 (this population surpassed 40,000 in 2002 but it has declined by 86% in 15 years)[47]. While they had intermittently been present, the number of seasonally resident leopard seals rose rapidly in the early 2000s at the largest breeding colony in the northern Antarctic peninsula region. Leopard seal numbers quickly increased by two orders of magnitude[39] as they preyed on seasonally available, easy-to-target pups. After the South Shetland Antarctic fur seal population crashed and this resource became scarce, leopard seal numbers decreased just as rapidly[48], although they are still observed at Cape Shirreff annually. The foraging species-level plasticity of leopard seals allowed them to maximally exploit this prey resource. However, there is insufficient behavioral and ecological data to explain how these solitary predators could respond so quickly at the population level to change their seasonal site fidelity.

Since 2008, a concerted effort was made to attach unique ID flipper tags to the most accessible hind flippers of leopard seal individuals hauled out at Cape Shirreff. Leopard seals in this area are often resighted repeatedly (seasonal residents; hereafter defined as "residents"), while a few are only seen once (hereafter seasonal "transients"). A large logistical effort is required to visually survey the > 10 km Cape Shirreff coastline for leopard seal individuals by foot, leading to incomplete tagging re-sight data. A seasonal survey of phocids, including leopard seals, is done weekly rather than daily at Cape Shirreff (census methods are described in detail by Woodman et al., 2024[49]). Therefore, tagged animals may be missed and this survey effort is likely further hindered by tag loss. Genomic analyses can be used to identify individuals over time and provide an assessment of tag loss, while allowing for the examination of social structure via the quantification of patterns of relatedness and inbreeding. Here, we leveraged more than ten years of sampling effort at Cape Shirreff and used genome-level data obtained via double digest restriction-site associated DNA (ddRAD) sequencing to confidently match individuals sampled over time while quantifying long-term individual site fidelity. This dataset also allowed us to conduct a detailed assessment of social structure via relatedness analyses among leopard seals detected at a single summering location. Based on opportunistic observations of tagged individuals at Cape Shirreff, we hypothesized that genomic matching would reveal long-term site fidelity for some individuals. We also hypothesized a general lack of social structure in leopard seals due to their solitary nature.

Results

To detect genetic matches and to characterize patterns of social structure and kinship, we ddRAD sequenced 88 leopard seal samples collected over a decade (2008 — 2018) from Cape Shirreff (Supplementary File A, Table S2). Most samples were obtained from animals fitted with flipper tags (n = 85, 96%), which allowed them to be roughly classified as either seasonal transients (n = 38) or residents (n = 47) according to census data. Most sampled animals were adult females (n = 70, 79%).

A total of 270,738,652 single-end 95 bp Illumina sequencing reads were generated and mapped to the leopard seal reference genome, with an average mapping rate of 96.5% ± 16.5. The aligned reads were then used to call a total of 465,523 raw SNPs. Following quality filtering, our dataset consisted of 79 samples representing 71 unique individuals (see results for genetic matches below) genotyped at 10,555 SNPs. The genotyping error rate estimated using genomic matches was low at 0.2% ± 0.3.

Detection of genetic matches/replicates

In our final sample dataset, we identified 10 individuals (9 females and 1 male) that were sampled more than once (Fig. 1). In turn, we failed to genetically match one individual that should have matched another based on flipper tag identification (biopsy sample ID 159,909 should have matched seal tag #100 based on field data). We attribute this to a likely mislabeling error in the laboratory or field.

The genetic matches revealed seasonal site fidelity at Cape Shirreff for several years (mean = 3.5 years; range 1–8 years) and detected seven flipper tag loss events. Specifically, four seals were unknowingly retagged and resampled in the field due to loss of the original flipper tag (Fig. 1). In one case, this happened twice to the same seal: original seal tag #48, was retagged as #84 two years later and retagged as #133 four years later. Incidental re-tagging/re-sampling occurred between one and seven years apart. Formal tag loss rates could not be accurately quantified for this dataset due to its small sample size and because the sampling was opportunistic.

Social structure and relatedness

The PCA analyses did not reveal any obvious clustering of individuals or differences between resident and transient seals, regardless of their sex (Fig. 2 c-f). Accordingly, the majority of leopard seals at Cape Shirreff were genetically unrelated (Fig. 2 a-b). However, both pairwise relatedness (GCTA) and kinship coefficient (KING) analyses revealed 3 closely related pairs of samples among the 71 individuals (Fig. 3a, b): seals tag # 397 (Fig. 4 a, b) and tag # 146 were identified as mother-daughter pair and a third female (tag # 127) was found to be the second-order relative (half-sibling, avuncular or grandparent–grandchild; $r_{xy} = 0.21$) of seal tag # 397 (these animals were identified as 3rd degree relatives according to KING coefficients). Finally, our analyses indicated a distant relationship between seals tag # 146 and tag # 127 ($r_{xy} = 0.095$), confirming kinship among three female leopard seals. All three seals (tag # 397, tag # 146 and tag # 127) shared the same mtDNA control region haplotype (GenBank accession # OQ451785.1; Bender et al., 2023), confirming their matrilineal kinship. Tissue sampling of the three related seals occurred in the month of January spanning five years. Notably, leopard seal tag # 127 was also found to be inbred (section below). Seals tag # 397 and tag # 127 were classified as residents, while seal tag # 146 was a transient (it is possible that she lost her tag).

Genetic diversity and inbreeding

Measures of multilocus heterozygosity (MLH) and inbreeding (Fhat 3) revealed that most leopard seals (n = 68; 95.77%) sampled at Cape Shirreff were not inbred; inbreeding was centered around zero; Fig. 5). The genomic inbreeding coefficient Fhat3 was negatively correlated with genome-wide heterozygosity (b = -0.7, $p < 0.001$, $R^2 = 0.77$). Three leopard seals had higher levels of inbreeding: "Untagged" (Fhat3 = 0.07), tag # 127 (Fhat3 = 0.1) and tag # 124 (Fhat3 = 0.17; Fig. 5). No differences in inbreeding levels were detected between resident and transient seals (Supplementary File B, Figure S1).

Discussion

Documenting patterns of site fidelity and social structure are crucial to our understanding of population dynamics, yet both are rarely studied in solitary mammals. This has led to a considerable gap in our understanding

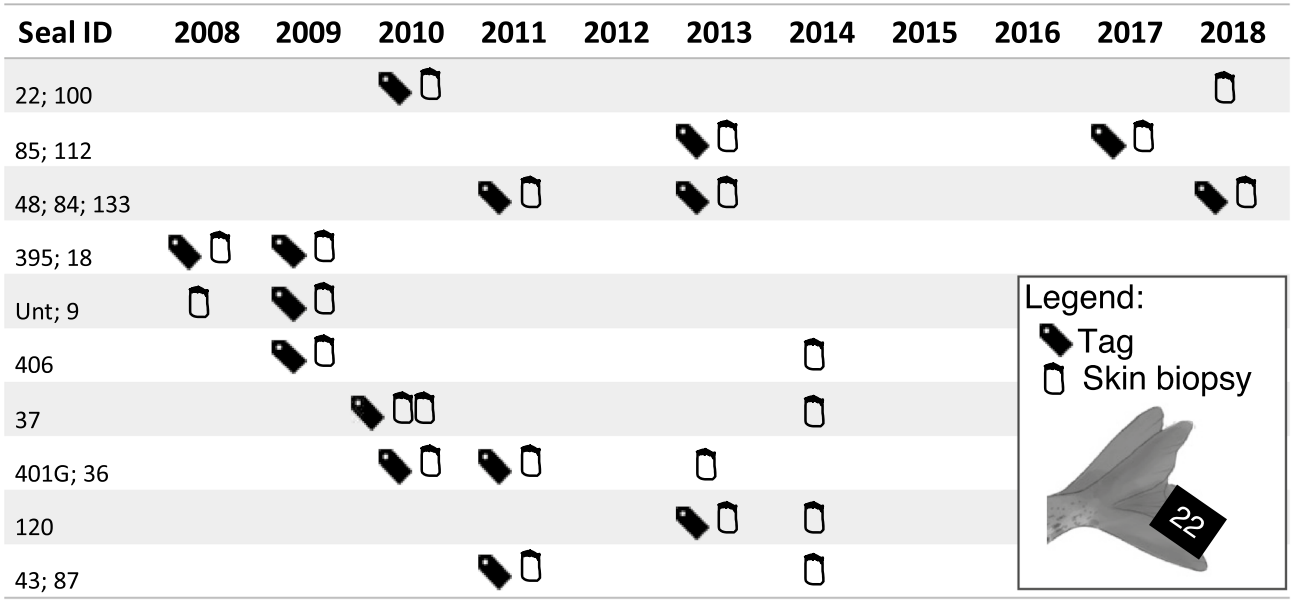

Fig. 1. A total of 79 leopard seal samples were analyzed using genomic data to determine identical matches. This analysis revealed several instances of tag loss, which are indicated by one seal having multiple Tag IDs listed. Tagging/ sampling events are shown in the order in which they occurred. All seals matched were identified as females, except seal tag # 120 (male).

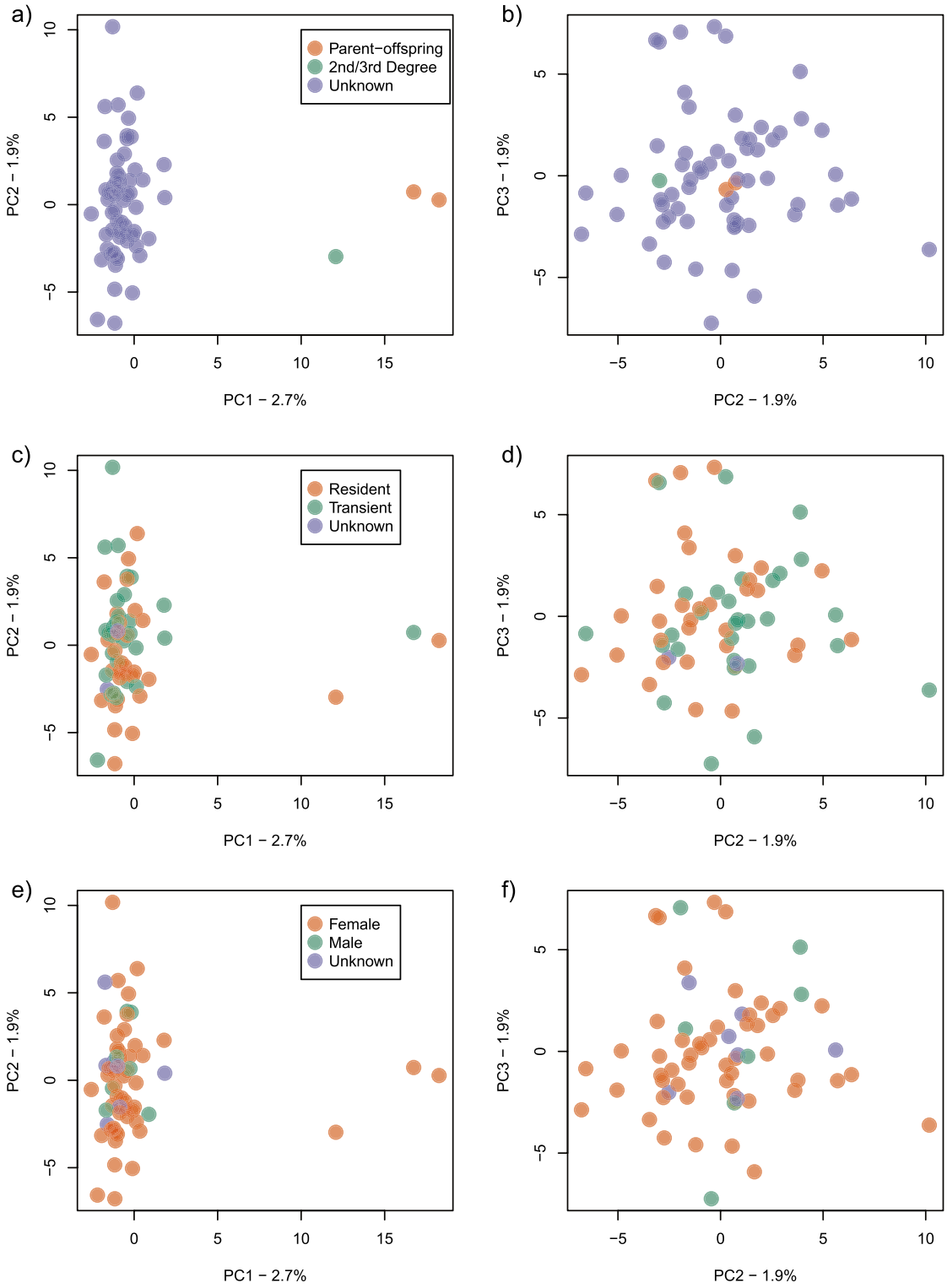

Fig. 2. Principal component analyses of individual leopard seal genomic variation. The first principal component reveals the three related individuals detected in this study (**a**-**b**). No significant portion of variance is explained by identifying leopard seals as females or males (**c**-**d**) and estimating their status as seasonal residents or transients (**e**–**f**).

of the potential ecological impacts of the behavior of large predators. A decade-long sampling and tagging effort at Cape Shirreff provided a rare opportunity to uncover key aspects of the behavior of leopard seals. Our effort revealed that long-term seasonal site fidelity was highly variable among individuals: although most seals were seasonal residents (57%), there was large number of transients (43%). Genomic matching revealed that among

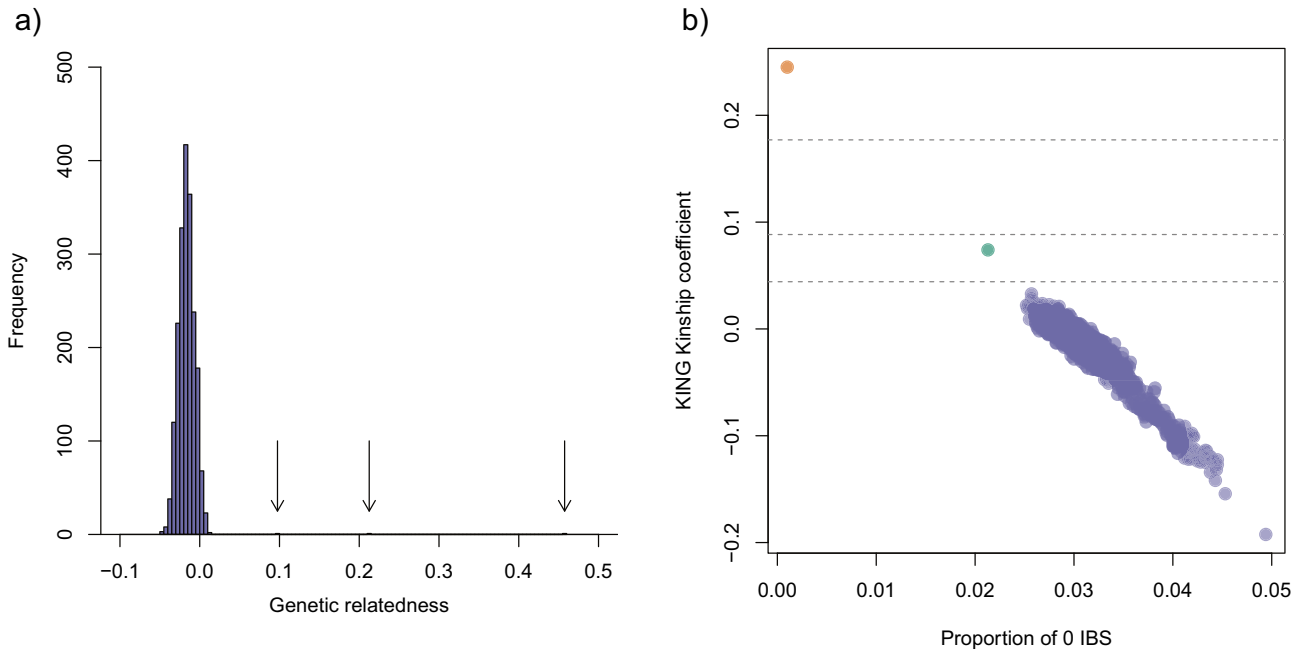

Fig. 3. Relatedness and kinship coefficients estimated for leopard seals sampled at Cape Shirreff. (**a**) Pairwise relatedness ($r_{xy;}$ GCTA) distribution indicates three relationships identified (arrows) among leopard seals; (**b**) KING kinship coefficient estimation for leopard seals (dots): colored dots indicate first (orange) and third (green) order relatives in the dataset, while purple dots indicate more distantly related pairs (greater than 3rd degree relatedness, which cannot be confidently inferred by KING) and unrelated individuals. Dashed horizontal lines show the threshold KING kinship coefficient values separating kinship classes.

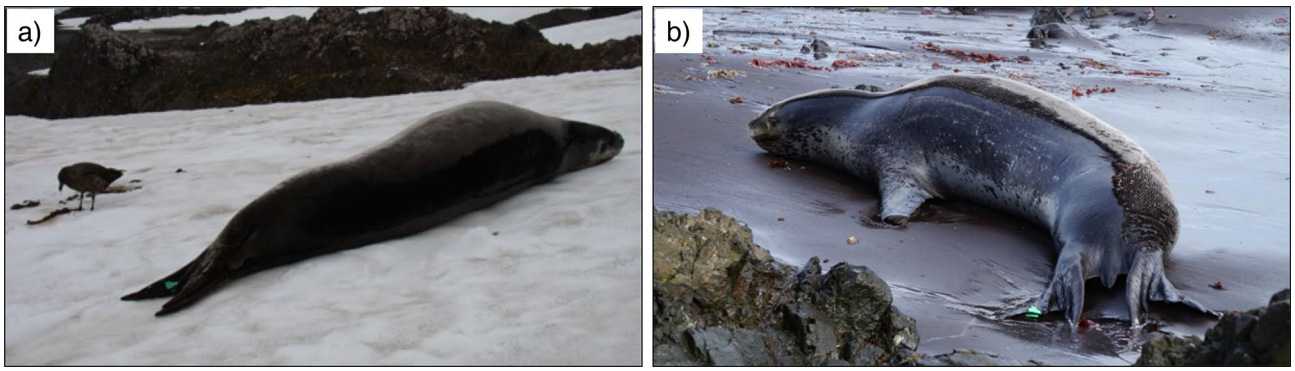

Fig. 4. Leopard seal tag # 397. Note the tag attached to the animal's hind flipper used for visual identification. (**a**) Seal tag # 397 photographed on Feb 01, 2010 at Cape Shirreff (photo credit: C. Bonin; (**b**) Seal tag # 397 photographed on Dec 21, 2021 at Cape Shirreff (photo credit: D. Krause).

the residents, seven individuals displayed remarkable site fidelity, including one leopard seal re-sampled three times in eight years. Genomic analyses also uncovered a case of transgenerational site-fidelity that spanned at least two generations of leopard seals. Leopard seals have had a key role in re-shaping trophic dynamics in the northern Antarctic peninsula and our results provide further behavioral context for their impacts on local prey populations.

The majority of leopard seals visiting Cape Shirreff were adult females returning to the area at least once during our study period; a few seals were re-sampled after nearly a decade. For seven individuals, our genomic dataset revealed substantially longer site fidelity to Cape Shirreff than was originally inferred from tag resighting data alone, indicating that tag loss is not negligible in this species as previously thought[50]. Flipper tags were generally resighted weekly, while the summer average haul-out period is less than one day[51]. Therefore, it is possible that resident animals were not seen and misclassified as transients, so this area may have more returning seals than we estimated. Regardless, our findings of long-term site fidelity at Cape Shirreff and the large proportion (79%) of adult females found there, contrast patterns observed at other locations such as Tasmania[52] and Bird Island, South Georgia, where transient juvenile leopard seals are more frequently observed[44,53,54]. At face value, this

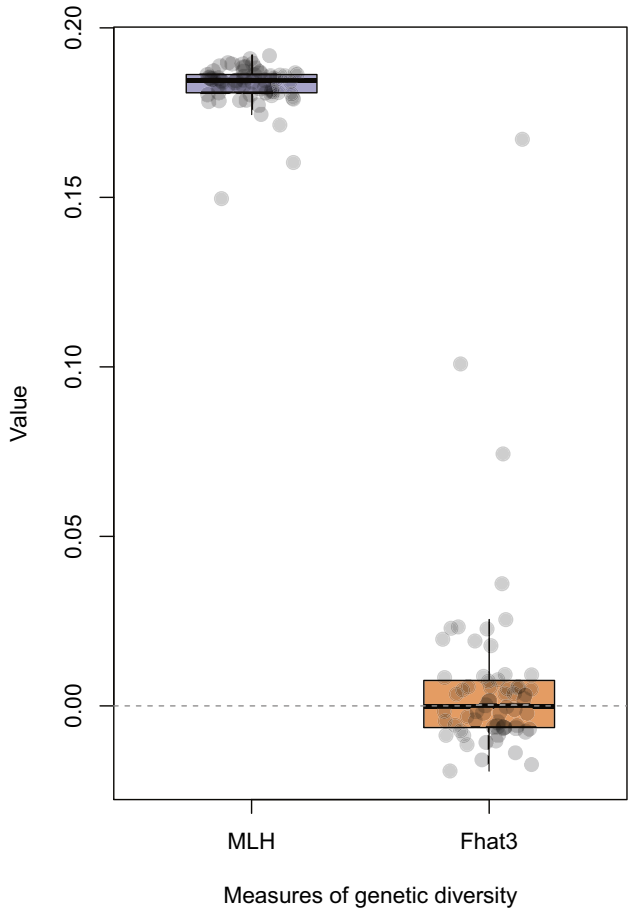

Fig. 5. Individual genetic diversity measures for leopard seals sampled at Cape Shirreff, Antarctica. Values for multilocus heterozygosity (MLH) and the inbreeding coefficient Fhat3. Each grey dot represents an individual.

is consistent with a pattern of post-breeding segregation that is possibly driven by intraspecific competition. Food-related aggressive encounters among leopard seals have been reported, including kleptoparasitism, when larger females steal prey from smaller conspecifics[43]. Another study at Cape Shirreff reported that the majority of leopard seals had wounds/scars on their bodies, which were likely the result of aggression; females had four times more scars than males[55]. This species is also sexually dimorphic, with females being at least 1.5 times larger than males[41] and feeding at higher trophic levels[56]. In fact, large females at Cape Shirreff dominate the consumption of Antarctic fur seal pups and when female densities are high, there are several lines of evidence to suggest that smaller conspecifics (e.g., males) are excluded from prime hunting areas near Antarctic fur seal colonies[56]. Notwithstanding, inference about post-breeding segregation should be made with caution because significant heterogeneity exists throughout the species' range. Notably, leopard seal males and females are both seasonal residents in Chile[40] where there is no clear evidence of age/sex segregation. Therefore, our findings lend further support to the argument that substantial regional variability exists in the behavior of this species.

Behavioral variability in leopard seals allows for high levels of specialization in some individuals. This has been consistently revealed in key aspects of leopard seal behavior, such as diet[57,58], prey handling/foraging[43,51,59] and movement[55]. The female leopard seal that we identified as a mother (tag # 397; Fig. 4 a, b) has been coincidentally observed in several studies at Cape Shirreff and epitomizes specialization. Seal tag # 397 is a large female, that grew considerably since it was first captured (2014 mass = 386 kg; 2014 length = 287 cm[56] ; 2018/19 mass range = 486/494 kg; 2018/19 standard length range = 298/ 300 cm[55]; Fig. 4 a, b). This seal is a specialist predator of Antarctic fur seal pups, feeding mostly at the top of food chain[57] with a diet that has varied little over ten years[58]. Primarily using ambush tactics, seal tag # 397 has an exceptional capture rate of 2.43 pups/hour and processes a pup carcass for consumption in approximately 10 min[43]. This leopard seal was tracked during the 2018 and 2019 austral fall when she travelled 512 km and 878 km respectively; this study also showed that seal tag # 397 does not typically dive deep (mean depth 29 ± 8 m, duration 3.0 ± 0.7 min[55]). In fact, while at Cape Shirreff for the summers, seal tag # 397 mostly follows the coastline, hunts at the two most dense Antarctic fur seal breeding beaches and processes her kills away from the shore [43]. It is not surprising that seal tag # 397, having optimized her hunting skills, has been returning annually for the summers and it is possible that social cues have led her kin to also identify Cape Shirreff as an ideal site for exploiting food resources.

Our discovery of three related female leopard seals at Cape Shirreff suggests that kin associations occur in this sparsely distributed, solitary species. In terrestrial, solitary carnivores such as brown bears, home range

overlap is positively correlated with relatedness[14,60] and black bear mothers (*Ursus americanus*) shift and reduce their home range to accommodate their daughters[61]. In polar bears (*Ursus maritimus*), females tend to show circannual movement patterns with site fidelity specific to each season[62], kin structuring is stronger among females[63], and offspring exhibit similar navigational patterns to their mothers[64]. One key caveat of comparing bears and leopard seals is the amount of maternal care: unlike bears, there is no evidence of post-weaning maternal care in leopard seals. Instead, lactation in phocids is typically short (e.g., 5–6 weeks in Weddell seals, *Leptonychotes weddellii*[65]; post-weaned pups tend to leave their natal area[66]). However, the first observations of leopard seals mating in the wild were only recently published[67], and there have been no studies regarding post-weaning dispersal, so inferences regarding the mechanisms that determine learned behaviors remain largely undefined.

Alternatively, it is plausible that Cape Shirreff's abundant prey resources (e.g., fur seals and penguins) attract leopard seals broadly, and the presence of related individuals there is coincidental. There is growing evidence that solitary carnivores are more tolerant of competition when resources are abundant. In these cases, increasing opportunities for social interactions exist but they are not necessarily associated with relatedness (e.g., carcass sharing in felids[32,68]). The high abundance of resources at Cape Shirreff attracts many animals from the region. Peak densities of leopard seals hauled out at Cape Shirreff between 2009 and 2014 were up to two orders of magnitude higher than those reported in other studies (22.7- 54.5 seals/nautical mile^2 vs. 0.01—0.521 seals/nautical mile^2, [43,49]). Nevertheless, leopard seals are a highly mobile[44,55] and comparatively abundant[69] circumpolar Antarctic top predator. The species is also broadly distributed across the Southern Ocean, occurring in low densities throughout the pack ice during the breeding season[70], so we argue that there is a low likelihood that the three related females detected in our study were coincidentally found at Cape Shirreff. Our data suggest that, although rare, social structure occurs in this solitary Southern Ocean species. However, further studies of leopard seal behavioral ecology, particularly regarding breeding, parental care and weaning are needed to understand the factors that influence post-breeding movements and foraging site fidelity and should help predict alterations of habitat use and species adaptability to environmental change, particularly ice loss.

Ice-dependent species such as Antarctic ice-breeding seals, including leopard seals, appear to have suffered recent alterations in their abundance and distribution[7,71]. Sea ice loss has accelerated over the past two decades[72,73] and although Antarctic krill (*Euphausia superba*) abundance trends remain uncertain[74] a southward shift in its distribution range[75] is impacting its dependent predator populations in the northern Antarctic peninsula. More specifically, the Antarctic fur seal population that consistently attracted leopard seals in high densities for the past 20 years has recently collapsed[47] and local penguin populations are also declining there[6,10,76]. These meso-predator populations no longer attract many apex predators, as evidenced by the lower density of leopard seals in this area during the past four years[40]. It is difficult to predict how mesopredator and apex predator populations will respond to drastic changes observed at lower levels of the food chain in this area. This dynamic region is currently undergoing a unique natural trophic cascade experiment and detailed knowledge of the ecology and behavior of its component species, such as the leopard seal, will be crucial for the effective management of this ecosystem.

Conclusion

Our genomic dataset revealed the presence of seasonal resident seals over nearly a decade, significantly extending the site fidelity duration obtained via tag resight data alone. Most importantly, it also revealed for the first time, social structure among leopard seals and foraging site fidelity to one location spanning at least two generations. The northern Antarctic peninsula ecosystem is undergoing dramatic changes in the availability of prey resources: large breeding aggregations of Antarctic fur seals are no longer available to leopard seals there. Further knowledge of leopard seal behavioral ecology will be necessary to help predict how this predator and their prey will respond to these changes.

Methods

Ethics statement

Leopard seal sampling and observations were conducted in austral summers from 2008 — 2018 in compliance with United States National Marine Fisheries Marine Mammal Protection Act permits 774–1847, 16,472, 20,599 and 25,786. Institutional approval for this work was obtained through the Institutional Animal Care and Use Committee of the National Marine Fisheries Service (IACUC numbers SWPI2011-02, 2014–03, and 2017–03). Adhering to both the Marine Mammal Protection Act and the IACUC protocols, ensured ethical treatment of animals, samples, and data. Furthermore, the following key components of the ARRIVE guidelines were scrutinized, monitored and periodically reported to the IACUC members and in permit reports and renewals. Each of the ARRIVE guidelines is specifically addressed by: (1) the experimental unit in this study is a single animal (collected in series and analyzed in aggregate), (2) samples sizes were determined by the opportunistic sampling of wildlife rather than by the controlled experimentation with laboratory animals, (3) 88 tissue samples were obtained and 9 individuals were excluded from genomic data analysis due to low sequencing yield or missing genotypes, (4) randomization was not needed because no treatment and control groups exist within these naturally occurring vertebrates that were opportunistically spotted and studied, (5) to prevent inadvertent bias, sample metadata (e.g., date of sampling, age class, sex) was masked/blinded during laboratory processing and from the coauthor that performed genomic analyses until the completion of the analyses (6) the disposition (e.g., continuous bloodwork, cell assessment, biomarker panels) of each sampled individual was not monitored beyond the period of sampling (< 10 min) because researchers minimize time and maximize distance in the presence of wildlife per permitting and IACUC protocols, (7) a priori assumptions about the number of samples needed to address the research questions of interest were not evaluated because of the designed opportunistic

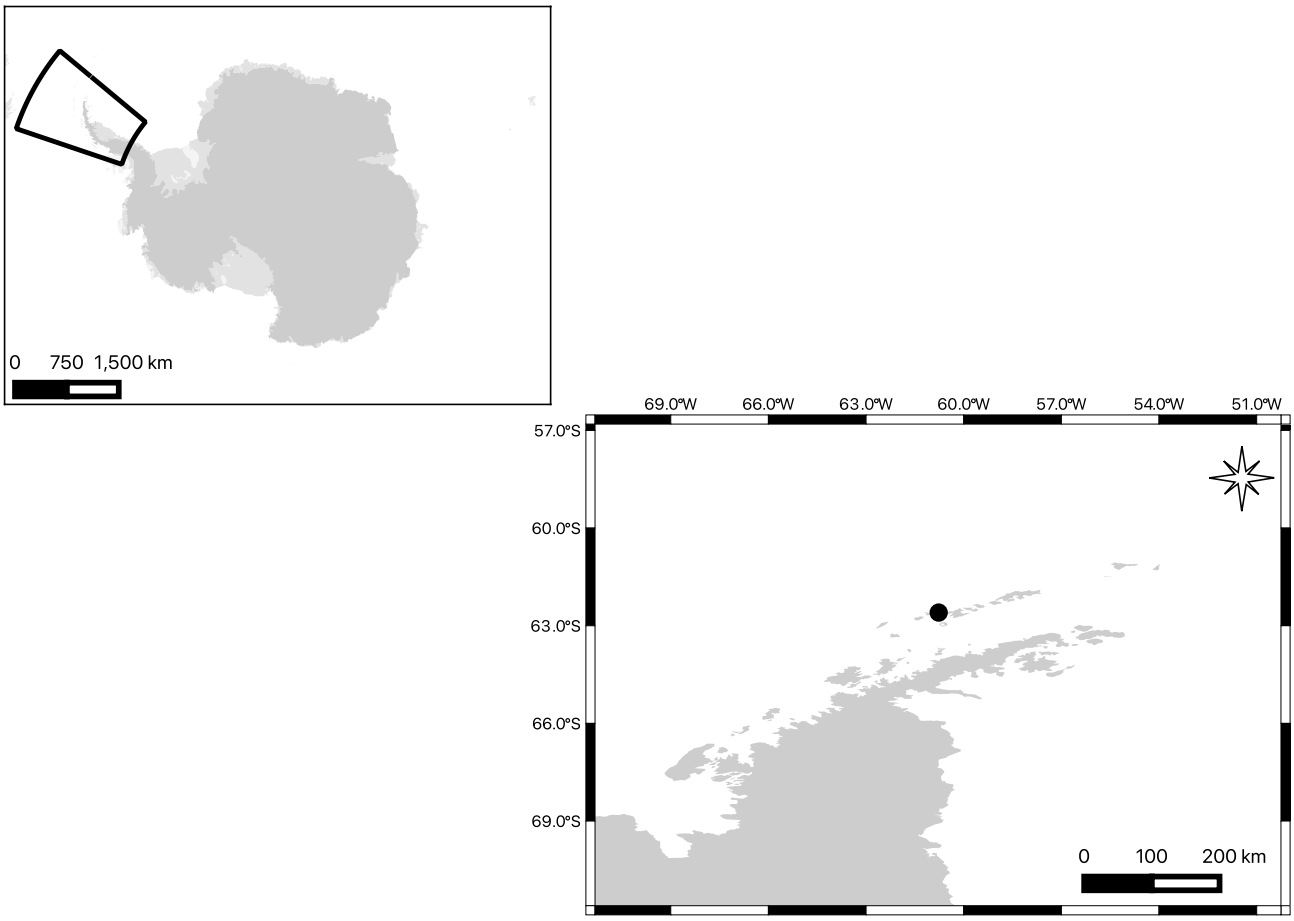

Fig. 6. Cape Shirreff (black dot in insert), in the northern Antarctic eninsula, where the leopard seals were sampled for this study.

sampling of large wild animals. All that could be sampled, were sampled, and still the sample sizes remain small compared to a pre-clinical study of laboratory animals. Therefore, statistical power and significance estimates were obtained post hoc using molecular data and software packages described elsewhere in the methods section, (8) all sampled individuals were leopard seals (*Hydrurga leptonyx*), and the strain/substrain category is not applicable, while the age, sex, and developmental stage of each individuals was collected (refer to Supplementary File A, TABLE S2), (9) leopard seals were opportunistically sampled to obtain a 2 mm-size skin sample. No anesthetics were used to reduce the pain and discomfort because these large animals (>300 kg) were not restrained. The tagging efforts were limited to a single attempt to minimize the handling time and reduce the stress experienced by each seal. No individual was sampled twice within the same week, although one animal was unknowingly sampled twice in the same season, (10) detailed descriptive summary statistics were calculated using genomic data including standardized measures of genetic diversity, such as inbreeding and heterozygosity. We confirm that the sampling and observations of leopard seals were performed in accordance with relevant guidelines and regulations. The study conducted did not include any in vivo animal experiments, or human clinical samples/data.

Leopard seal tagging and sampling

Leopard seals were tagged and tissue sampled at Cape Shirreff (62°28S; 60°47'W), Livingston Island, in the northern Antarctic peninsula (Fig. 6). The U.S. Antarctic Marine Living Resources Program (U.S. AMLR) has conducted long-term monitoring of pinniped and penguin populations at Cape Shirreff for more than 30 years. Focused on Antarctic fur seals, gentoo (*Pygoscelis papua*) and chinstrap (*Pygoscelis antarctica*) penguins, U.S. AMLR provides essential data for ecosystem indicator species identified by Commission for the Conservation of Antarctic Marine Living Resources (CCAMLR; [43]). Other species, including leopard seals, have been part of ancillary studies and their sampling has been more opportunistic. When researchers observe an unidentified leopard seal on shore, attempts are made to identify, flipper tag, and sample the individual. Additional dedicated studies involving captures have also been conducted at Cape Shirreff during select seasons[43,55].

Cape Shirreff is a peninsula that is ice-free during austral summers and has a permanent glacier on its southern edge. The area has several beaches surrounded by rocky outcrops. Leopard seals are found there in the austral summers (Nov-Mar), with the highest density of seals recorded in January and February[43]. Leopard seals are usually observed lying on beaches alone or swimming in areas occupied by penguins or Antarctic fur seals.

When leopard seals are hauled out on land it is possible to approach them on foot, without visibly disturbing them and apply a flipper tag. Flipper tagging consisted of applying an ID-tag (Dalton Jumbo-rototag or Allflex ear tags) to the outer inter-digital webbing of the seal's most accessible hind flipper while the seal was resting on shore. For this study, tissues from leopard seals were collected by two routes: by collecting the waste tissue that remained in the pliers used for fixation of a tag, or by using a 2 mm sterile biopsy punch to obtain a small skin plug (Miltex). Samples were stored in 95% ETOH and then kept frozen at -20 °C until processing. Once tagged (e.g., Fig. 4 a, b), individual leopard seal resightings were opportunistically recorded in each subsequent year. Resightings were also systematically recorded during weekly phocid censuses during the summers, which consisted of trained observers counting all phocids on shore at Cape Shirreff and categorizing them by species and age class. These censuses are described in detail in Woodman et al., 2024[49] and were conducted during this study's period. Seal tag IDs were identified using handheld binoculars.

Using tag resighting data obtained via censuses, we categorized leopard seals as either residents (animals tagged at Cape Shirreff and resighted at least once across years) or transients (animals that were tagged at Cape Shirreff but never resighted). Note that both tagging and tag resight data were opportunistic and some of the "transient" seals may still have been present at Cape Shirreff but not observed by the researchers. We therefore used this classification to guide only one aspect of our data analyses, which was to verify whether the groups "transient" or "resident" help explain genetic data variance.

Sample preparation and ddRAD sequencing

Leopard seal genomic DNA was isolated from 89 skin samples including one technical replicate (DNA was re-extracted and added to the plate). We used a commercial kit (DNeasy tissue and Blood kit, Qiagen) and assessed sample DNA quantity and quality with a Nanodrop spectrophotometer (ThermoFisher Scientific) and by running a subset of the samples on a 2% agarose gel. After selecting high quality DNA extracts, we submitted ~ 1,000 ng of DNA from each sample to Floragenex (Beaverton, Oregon) for ddRAD library preparation and sequencing.

Genomic DNA was digested with the restriction endonucleases PstI and MseI and processed into SBG libraries according to previously described methods[77]. Briefly, ~ 125 ng of genomic DNA was digested for 120 min at 37 °C in a 20 µL reaction with 20 units (U) of *PstI* and 2.75 U of *MseI* (New England Biolabs). After digestion, the samples were heat-inactivated for 10 min at 80 °C followed by the addition of 2.5 µL of 1 µM *P1* and 0.1 µL of 250 µM *MseI* adapters. *PstI P1* adapters each contained a unique multiplex sequence index (barcode; first five nucleotides). *P1* and *P2* adaptors were added to each sample along with 1 µL RL Buffer A (Keygene), 0.83 µL RL Buffer B (Keygene), 0.5 µL 10 mM ATP, T4 DNA Ligase (high concentration, Enzymatics, Inc), 0.3125 U MseI, 0.25 U PstI in a final reaction volume of 25 µL, which was then incubated at 37 °C for 180 min. Next, the samples were diluted 1:10 in water and 2.5 µL of this product was used in a PCR amplification using the following reagents: 10 × PCR Buffer 1 (Applied Biosystems), 0.2 U AmpliTaq DNA Polymerase (Applied Biosystems), 0.1 µL 20 nM dNTP mix, 0.3 µL of 50 ng/µL *MseI* primer, 0.05 µL of *PstI* primer, and 6.01 µL water. The following PCR cycling protocol was employed: 2 min 72 °C, followed by 13 cycles of: 30 s 94 °C; 2 min 67 °C, decreasing -0.7 °C per cycle; 2 min 72 °C and then 37 cycles of: 30 s 94 °C; 2 min 58 °C; 2 min 72 °C and a final hold at 4 °C. The PCR product obtained from each sample was pooled and 125 µL was purified with a MinElute Enzymatic Reaction Cleanup Kit (Qiagen), eluted and run on a 1.5% agarose gel. DNA bands appearing at 300 bp to 800 bp were excised from the gel and purified with the MinElute Gel Extraction Kit (Qiagen).

The used ddRAD protocol has been extensively described elsewhere (e.g.,[78–80]). In brief, the excised DNA was subjected to restriction digestion with *SgrAI*. Then, individual barcodes and sequence adapters were ligated to the genomic DNA. The barcoded samples were pooled, and fragments were sequenced to obtain 100 bp single end reads in a HiSeq 2000 platform (Illumina). The reads were de-multiplexed and barcodes were trimmed resulting in final DNA fragment lengths of 95 bp.

ddRAD data processing

We determined the quality of the demultiplexed sequencing reads using FastQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/). Three samples were excluded from further analyses due to low numbers of reads (< 1,000,000, Supplementary File A, Table S1). The remaining sequences were then aligned to the leopard seal reference genome (NCBI GenBank accession #JBJQNM000000000;[81]) using BWA MEM v0.7.13[82] with default parameters. The resulting alignment files were used as input within the ref_map.pl tool of the Stacks pipeline[83] for SNP calling and genotyping. Next, we filtered the raw genotypes to retain only SNP calls with genotype quality and depth of coverage ≥ 10 using VCFTools[84]. The remaining SNP genotypes were further filtered to exclude SNPs with genotyping rate below 90% and depth of coverage larger than twice the mean coverage of the dataset using VCFTools. The latter step was implemented to remove potentially paralogous loci. Furthermore, we used PLINK[85] to remove SNPs with minor allele frequency < 0.01 and any loci showing significant departures from Hardy–Weinberg Equilibrium using a p-value cut-off of 0.001 after applying mid-p correction[86]. Importantly, the technical replicate was not used to calculate summary statistics used during quality filtering of SNPs. As a final filtering step, we removed any individual with a genotyping rate lower than 80%. Next, we used the software KING[87] to determine if any genomic matches were present within our dataset. As the presence of duplicated samples within a dataset can bias the calculation of summary statistics used for SNP filtering, such as minor allele frequency, we removed all duplicated genotypes (by randomly selecting one) from the raw unfiltered SNP dataset before repeating our filtering strategy. Finally, we used data from all genetically matched individuals, to estimate the genotyping error rate within our dataset.

Social structure (kinship)

To test for population structure within our sample of individuals from Cape Shirreff, we subjected our SNP dataset to a principal component analysis (PCA) using the R package adegenet[88]. We searched for patterns by

dividing samples based on the approximate classification of resident vs. transient individuals as well as sex. Next, we used KING[87] to infer whether any related individuals, up to and including 3rd degree relatives, were present within our sample set. This software returns the KING-robust kinship coefficient, which is independent of sample composition or population structure, and allows for the confident detection of parent–offspring pairs, full- and half-siblings, and third-degree relatives. In addition, we used GCTA[89] to estimate pairwise relatedness values (r_{xy}) between all pairs of samples.

Individual genetic diversity

We estimated each individual leopard seals' genome-wide level of inbreeding by calculating multi-locus heterozygosity (MLH) using the R package inbreedR[90] and the inbreeding coefficient Fhat3[89] using PLINK.

Data availability

The code used to analyze the ddRAD sequencing data and for downstream analysis is available at: https://github.com/DavidVendrami/LeopardSeals_ddRAD. Raw sequence files and sample metadata were deposited in NCBI SRA under BioProject ID: PRJNA1234666 and BioSample accession numbers SAMN47306572—SAMN47306651.

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References

1. Estes, J. A., Tinker, M. T., Williams, T. M. & Doak, D. F. Killer whale predation on sea otters linking oceanic and nearshore ecosystems. *Science* **282**, 473–476 (1998).
2. Estes, J. A. et al. Trophic downgrading of planet Earth. *Science* **333**, 301–306 (2011).
3. Estes, J. A., Heithaus, M., McCauley, D. J., Rasher, D. B. & Worm, B. Megafaunal impacts on structure and function of ocean ecosystems. *Annu. Rev. Environ. Resour.* **41**, 83–116 (2016).
4. Croxall, J. P. Southern ocean environmental changes: effects on seabird, seal and whale populations. *Philos. Trans. R. Soc. B: Biol. Sci.* **338**, 319–328 (1992).
5. Ducklow, H. W. et al. Marine pelagic ecosystems: the west Antarctic Peninsula. *Philos. Trans. R. Soc. B: Biol. Sci.* **362**, 67–94 (2007).
6. Hinke, J. T., Salwicka, K., Trivelpiece, S. G., Watters, G. M. & Trivelpiece, W. Z. Divergent responses of Pygoscelis penguins reveal a common environmental driver. *Oecologia* **153**, 845–855 (2007).
7. Forcada, J. et al. Responses of Antarctic pack-ice seals to environmental change and increasing krill fishing. *Biol. Cons.* **149**, 40–50 (2012).
8. Ducklow, H. W. et al. West Antarctic Peninsula: an ice-dependent coastal marine ecosystem in transition. *Oceanography* **26**, 190–203 (2013).
9. Forcada, J. et al. Ninety years of change, from commercial extinction to recovery, range expansion and decline for Antarctic fur seals at South Georgia. *Glob. Change Biol.* **29**, 6867–6887 (2023).
10. Trivelpiece, W. Z. et al. Variability in krill biomass links harvesting and climate warming to penguin population changes in Antarctica. *Proc. Natl. Acad. Sci.* **108**, 7625–7628 (2011).
11. Pardo, D. et al. Additive effects of climate and fisheries drive ongoing declines in multiple albatross species. *Proc. Natl. Acad. Sci.* **114**, E10829–E10837 (2017).
12. Hindell, M. A. et al. Decadal changes in habitat characteristics influence population trajectories of southern elephant seals. *Glob. Change Biol.* **23**, 5136–5150 (2017).
13. Watters, G. M., Hinke, J. T. & Reiss, C. S. Long-term observations from Antarctica demonstrate that mismatched scales of fisheries management and predator-prey interaction lead to erroneous conclusions about precaution. *Sci. Rep.* **10**, 2314 (2020).
14. Olejarz, A. et al. Ain't nothing like family—female brown bears share their home range with relatives. *Diversity* **14**, 41 (2022).
15. Makuya, L. & Schradin, C. Costs and benefits of solitary living in mammals. *J. Zool.* **323**, 9–18 (2024).
16. Smith, J. E., Lehmann, K. D., Montgomery, T. M., Strauss, E. D. & Holekamp, K. E. Insights from long-term field studies of mammalian carnivores. *J. Mammal.* **98**, 631–641 (2017).
17. Wakefield, E. D. et al. Long-term individual foraging site fidelity—why some gannets don't change their spots. *Ecology* **96**, 3058–3074 (2015).
18. Arthur, B. et al. Return customers: Foraging site fidelity and the effect of environmental variability in wide-ranging Antarctic fur seals. *PLoS ONE* **10**, e0120888 (2015).
19. Knox, T. C., Baylis, A. M. & Arnould, J. P. Foraging site fidelity in male Australian fur seals. *Mar. Biol.* **165**, 1–12 (2018).
20. Authier, M., Bentaleb, I., Ponchon, A., Martin, C. & Guinet, C. Foraging fidelity as a recipe for a long life: foraging strategy and longevity in male southern elephant seals. *PLoS ONE* **7**, e32026 (2012).
21. McIntyre, T. et al. Tracking the foraging migrations of Marion Island southern elephant seals (*Mirounga leonina*) during their first year of life. *Mar. Mamm. Sci.* **40**, e13078 (2024).
22. Cameron, M. F., Siniff, D. B., Proffitt, K. M. & Garrott, R. A. Site fidelity of Weddell seals: the effects of sex and age. *Antarct. Sci.* **19**, 149–155 (2007).
23. Votier, S. C. et al. Effects of age and reproductive status on individual foraging site fidelity in a long-lived marine predator. *Proc. R. Soc. B: Biol. Sci.* **284**, 20171068 (2017).
24. Fay, F. H. Odobenus rosmarus. *Mamm. Species.* **238**, 1–7 (1985).
25. Guiry, E. J., James, M., Cheung, C. & Royle, T. C. Four millennia of long-term individual foraging site fidelity in a highly migratory marine predator. *Commun. Biol.* **5**, 368 (2022).
26. Abrahms, B. et al. Climate mediates the success of migration strategies in a marine predator. *Ecol. Lett.* **21**, 63–71 (2018).
27. Augé, A., Chilvers, B., Moore, A. & Davis, L. Importance of studying foraging site fidelity for spatial conservation measures in a mobile predator. *Anim. Conserv.* **17**, 61–71 (2014).
28. Biard, V., Nykänen, M., Niemi, M. & Kunnasranta, M. Extreme moulting site fidelity of the Saimaa ringed seal. *Mamm. Biol.* **102**, 1483–1495 (2022).
29. Wolf, J. B. & Trillmich, F. Beyond habitat requirements: individual fine-scale site fidelity in a colony of the Galapagos sea lion (*Zalophus wollebaeki*) creates conditions for social structuring. *Oecologia* **152**, 553–567 (2007).
30. Wolf, J. B., Mawdsley, D., Trillmich, F. & James, R. Social structure in a colonial mammal: unravelling hidden structural layers and their foundations by network analysis. *Anim. Behav.* **74**, 1293–1302 (2007).
31. Elbroch, L. M. & Quigley, H. Social interactions in a solitary carnivore. *Curr. Zool.* **63**, 357–362 (2017).
32. Elbroch, L. M., Levy, M., Lubell, M., Quigley, H. & Caragiulo, A. Adaptive social strategies in a solitary carnivore. *Sci. Adv.* **3**, e1701218 (2017).

33. Graw, B., Kranstauber, B. & Manser, M. B. Social organization of a solitary carnivore: spatial behaviour, interactions and relatedness in the slender mongoose. *R. Soc. Open Sci.* **6**, 182160 (2019).
34. Hansen, J. E., Hertel, A. G., Frank, S. C., Kindberg, J. & Zedrosser, A. Social environment shapes female settlement decisions in a solitary carnivore. *Behav. Ecol.* **33**, 137–146 (2022).
35. Holekamp, K. E. & Sawdy, M. A. The evolution of matrilineal social systems in fissiped carnivores. *Philos. Trans. R. Soc. B* **374**, 20180065 (2019).
36. Rogers, T. *Encyclopedia of marine mammals* (Academic Press, 2017).
37. Aguayo-Lobo, A. *et al.* Presence of the leopard seal, *Hydrurga leptonyx* (De Blainville, 1820), on the coast of Chile: an example of the Antarctica-South America connection in the marine environment. (2011).
38. van der Linde, K. et al. A review of leopard seal (*Hydrurga leptonyx*) births and pups using a standardised age-class classification system. *Polar Biol.* **45**, 1193–1209 (2022).
39. Shaughnessy, P. D., Tomo, I., Gibbs, S. E., Kemper, C. M. & Stemmer, D. Records of leopard seals *Hydrurga leptonyx* ashore in South Australia, 2017–2022. *Aust. Mammal.* **46**, NULL-NULL (2023).
40. Borras-Chavez, R. et al. Occurrence, residency, and habitat characterization of leopard seals in Chile. *Front. Ecol. Evol.* **12**, 1448098 (2024).
41. Southwell, C., Kerry, K., Ensor, P., Woehler, E. J. & Rogers, T. The timing of pupping by pack-ice seals in East Antarctica. *Polar Biol.* **26**, 648–652 (2003).
42. Ainley, D. G., Ballard, G., Karl, B. J. & Dugger, K. M. Leopard seal predation rates at penguin colonies of different size. *Antarct. Sci.* **17**, 335–340 (2005).
43. Krause, D. J., Goebel, M. E., Marshall, G. J. & Abernathy, K. Novel foraging strategies observed in a growing leopard seal (*Hydrurga leptonyx*) population at Livingston Island Antarctic Peninsula. *Animal Biotelemetry* **3**, 1–14 (2015).
44. Staniland, I. J., Ratcliffe, N., Trathan, P. N. & Forcada, J. Long term movements and activity patterns of an Antarctic marine apex predator: The leopard seal. *PLoS ONE* **13**, e0197767 (2018).
45. Laws, R. *Seals* (Academic Press, 1984).
46. Boveng, P. L., Hiruki, L. M., Schwartz, M. K. & Bengtson, J. L. Population growth of Antarctic fur seals: limitation by a top predator, the leopard seal?. *Ecology* **79**, 2863–2877 (1998).
47. Krause, D. J., Bonin, C. A., Goebel, M. E., Reiss, C. S. & Watters, G. M. The rapid population collapse of a key marine predator in the northern Antarctic Peninsula endangers genetic diversity and resilience to climate change. *Front. Mar. Sci.* **8**, 796488 (2022).
48. Krause, D. J. et al. Evaluating threats to South Shetland Antarctic fur seals amidst population collapse. *Mammal Rev.* **54**, 30–46 (2024).
49. Woodman, S. M. et al. CS-PHOC: weekly census counts of Southern Ocean phocids at Cape Shirreff Livingston Island. *Sci. Data* **11**, 895 (2024).
50. Walker, T. et al. Seasonal occurrence and diet of leopard seals (*Hydrurga leptonyx*) at Bird Island South Georgia. *Antarctic Sci.* **10**, 75–81 (1998).
51. Krause, D. J., Goebel, M. E., Marshall, G. J. & Abernathy, K. Summer diving and haul-out behavior of leopard seals (*Hydrurga leptonyx*) near mesopredator breeding colonies at Livingston Island Antarctic Peninsula. *Mar. Mammal Sci.* **32**, 839–867 (2016).
52. Rounsevell, D. & Pemberton, D. The status and seasonal occurrence of leopard seals, *Hydrurga leptonyx* Tasmanian waters. *Aust. Mammal.* **17**, 97–102 (1994).
53. Jessopp, M., Forcada, J., Reid, K., Trathan, P. & Murphy, E. Winter dispersal of leopard seals (*Hydrurga leptonyx*): environmental factors influencing demographics and seasonal abundance. *J. Zool.* **263**, 251–258 (2004).
54. Forcada, J. & Robinson, S. L. Population abundance, structure and turnover estimates for leopard seals during winter dispersal combining tagging and photo-identification data. *Polar Biol.* **29**, 1052–1062 (2006).
55. Kienle, S. S. et al. Plasticity in the morphometrics and movements of an Antarctic apex predator, the leopard seal. *Front. Mar. Sci.* **9**, 976019 (2022).
56. Krause, D. J., Goebel, M. E. & Kurle, C. M. Leopard seal diets in a rapidly warming polar region vary by year, season, sex, and body size. *BMC Ecol.* **20**, 1–15 (2020).
57. Sperou, E. S. et al. Large and In Charge: Cortisol levels vary with sex, diet, and body mass in an Antarctic predator, the leopard seal. *Front. Mar. Sci.* **10**, 1179236 (2023).
58. Sperou, E. S. et al. Individual Specialization in a Generalist Apex Predator: The Leopard Seal. *Ecol. Evol.* **15**, e71593 (2025).
59. Krause, D. J. & Rogers, T. L. Food caching by a marine apex predator, the leopard seal (*Hydrurga leptonyx*). *Can. J. Zool.* **97**, 573–578 (2019).
60. Støen, O.-G., Bellemain, E., Sæbø, S. & Swenson, J. E. Kin-related spatial structure in brown bears *Ursus arctos*. *Behav. Ecol. Sociobiol.* **59**, 191–197 (2005).
61. Rogers, L. L. Factors influencing dispersal in the black bear. *Mammalian dispersal patterns: The effects of social structure on population genetics. University of Chicago Press, Chicago, Illinois, USA*, 75–84 (1987).
62. Mauritzen, M., Derocher, A. E. & Wiig, Ø. Space-use strategies of female polar bears in a dynamic sea ice habitat. *Can. J. Zool.* **79**, 1704–1713 (2001).
63. Zeyl, E., Aars, J., Ehrich, D. & Wiig, Ø. Families in space: relatedness in the Barents Sea population of polar bears (*Ursus maritimus*). *Mol. Ecol.* **18**, 735–749 (2009).
64. Derocher, A. E. & Stirling, I. Distribution of polar bears (*Ursus maritimus*) during the ice-free period in western Hudson Bay. *Can. J. Zool.* **68**, 1395–1403 (1990).
65. Wheatley, K. E., Bradshaw, C. J., Davis, L. S., Harcourt, R. G. & Hindell, M. A. Influence of maternal mass and condition on energy transfer in Weddell seals. *Journal of Animal Ecology*, 724–733 (2006).
66. Burns, J., Castellini, M. & Testa, J. Movements and diving behavior of weaned Weddell seal (*Leptonychotes weddellii*) pups. *Polar Biol.* **21**, 23–36 (1999).
67. Kienle, S. S. et al. First paired observations of sexual behavior and calls in wild leopard seals. *Polar Biol.* **47**, 1025–1037 (2024).
68. López-Bao, J. V., Rodríguez, A. & Alés, E. Field observation of two males following a female in the Iberian lynx (*Lynx pardinus*) during the mating season. (2008).
69. Bender, A. N. et al. Genetic diversity and demographic history of the leopard seal: A Southern Ocean top predator. *PLoS ONE* **18**, e0284640 (2023).
70. Southwell, C. et al. Uncommon or cryptic? Challenges in estimating leopard seal abundance by conventional but state-of-the-art methods. *Deep Sea Res. Part I* **55**, 519–531 (2008).
71. Hückstädt, L. A. et al. Projected shifts in the foraging habitat of crabeater seals along the Antarctic Peninsula. *Nat. Clim. Chang.* **10**, 472–477 (2020).
72. Parkinson, C. L. & Cavalieri, D. J. Antarctic sea ice variability and trends, 1979–2010. *Cryosphere* **6**, 871–880 (2012).
73. Turner, J., Hosking, J. S., Bracegirdle, T. J., Marshall, G. J. & Phillips, T. Recent changes in Antarctic sea ice. *Philosophical Trans. R. Soc. A: Math., Phys. Eng. Sci.* **373**, 20140163 (2015).
74. Warwick-Evans, V., Fielding, S., Reiss, C., Watters, G. & Trathan, P. N. Estimating the average distribution of Antarctic krill *Euphausia superba* at the northern Antarctic Peninsula during austral summer and winter. *Polar Biol.* **45**, 857–871 (2022).
75. Atkinson, A. et al. Krill (*Euphausia superba*) distribution contracts southward during rapid regional warming. *Nat. Clim. Chang.* **9**, 142–147 (2019).

76. Lynch, H. J., Naveen, R., Trathan, P. N. & Fagan, W. F. Spatially integrated assessment reveals widespread changes in penguin populations on the Antarctic Peninsula. *Ecology* **93**, 1367–1377 (2012).
77. Truong, H. T. et al. Sequence-based genotyping for marker discovery and co-dominant scoring in germplasm and populations. *PLoS ONE* **7**, e37565 (2012).
78. Baird, N. A. et al. Rapid SNP discovery and genetic mapping using sequenced RAD markers. *PLoS ONE* **3**, e3376 (2008).
79. Hohenlohe, P. A. et al. Population genomics of parallel adaptation in threespine stickleback using sequenced RAD tags. *PLoS Genet.* **6**, e1000862 (2010).
80. Lozier, J. Revisiting comparisons of genetic diversity in stable and declining species: assessing genome-wide polymorphism in N orth A merican bumble bees using RAD sequencing. *Mol. Ecol.* **23**, 788–801 (2014).
81. Canitz, J. et al. Reference genome of the leopard seal (*Hydrurga leptonyx*), a Southern Ocean apex predator. *Front. Genet.* **16**, 1561273 (2025).
82. Li, H. Aligning sequence reads, clone sequences and assembly con-tigs with BWA-MEM. *Genomics* **1303** (2013).
83. Rochette, N. C., Rivera-Colón, A. G. & Catchen, J. M. Stacks 2: Analytical methods for paired-end sequencing improve RADseq-based population genomics. *Mol. Ecol.* **28**, 4737–4754 (2019).
84. Danecek, P. et al. The variant call format and VCFtools. *Bioinformatics* **27**, 2156–2158 (2011).
85. Purcell, S. et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am. J. Human Genet.* **81**, 559–575 (2007).
86. Graffelman, J. & Moreno, V. The mid p-value in exact tests for Hardy-Weinberg equilibrium. *Stat. Appl. Genet. Mol. Biol.* **12**, 433–448 (2013).
87. Manichaikul, A. et al. Robust relationship inference in genome-wide association studies. *Bioinformatics* **26**, 2867–2873 (2010).
88. Jombart, T. adegenet: a R package for the multivariate analysis of genetic markers. *Bioinformatics* **24**, 1403–1405 (2008).
89. Yang, J., Lee, S. H., Goddard, M. E. & Visscher, P. M. GCTA: a tool for genome-wide complex trait analysis. *Am. J. Human Genet.* **88**, 76–82 (2011).
90. Stoffel, M. A. et al. inbreedR: an R package for the analysis of inbreeding based on genetic markers. *Meth. Ecol. Evol.* **7**, 1331–1339 (2016).

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Author contributions

CAB: study design, molecular data acquisition, writing manuscript, funding acquisition DLJV: data analyses, results interpretation, writing manuscript MEG: study design, sample acquisition, ecological data acquisition, review/editing manuscript SSK: review/editing manuscript, funding acquisition JIH: study design, results interpretation, review/ editing manuscript, funding acquisition DJK: study design, sample acquisition, ecological data acquisition, writing manuscript.

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Declarations

Competing interests

The authors declare no competing interests.

Additional information

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