Kinship and association in a highly social apex predator population, killer whales at Marion Island

Ryan R. Reisinger, Charlene Beukes (née Janse van Rensburg), A. Rus Hoelzel, P.J. Nico de Bruyn

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AUTHORS

Ryan R. Reisinger[1,2*], Charlene Beukes (née Janse van Rensburg)[1], A. Rus Hoelzel[3], P.J. Nico de Bruyn[1]

[1] Mammal Research Institute, Department of Zoology and Entomology, University of Pretoria, South Africa

[2] Department of Zoology, Nelson Mandela Metropolitan University, South Africa

[3] School of Biological and Biomedical Sciences, Durham University, United Kingdom

[*]Corresponding author:

ryan.r.reisinger@gmail.com

Mammal Research Institute, Department of Zoology and Entomology, University of Pretoria, Private Bag X20, Hatfield 0028, Pretoria, South Africa

and

Department of Zoology, Nelson Mandela Metropolitan University, PO Box 77000, Port Elizabeth 6031, South Africa

ABSTRACT

Social structure is a core element of population biology, influenced by intrinsic and environmental factors. Intra-taxon comparisons of social organization are useful in elucidating the role of such ecological determinants of sociality. Killer whales *Orcinus orca* are widely-distributed, social delphinids with diverse morphology, diet, behavior and genetics, but few studies have quantitatively examined social structure in this species. We used 7 years of individual identification data on killer whales at Marion Island, Southern Ocean, to calculate the half-weight association index among 39 individuals, creating a weighted association network. There were long-term associations between individuals, though associations were dynamic over time. We defined 8 social modules using a community detection algorithm and these typically contained 3 individuals of various ages and sexes. Pairwise genetic relatedness among 20 individuals was not significantly correlated with association index. Individuals were on average more related within than between social modules, but social modules contained related as well as unrelated individuals. Likely parent pairs of six individuals indicated mating between social modules.

KEYWORDS

sociality; socioecology; social structure; network; group; relatedness; predators; delphinids

INTRODUCTION

Living in groups must represent a fitness advantage to persist. Various social structures result from the optimization of the costs and benefits of group living, within a varying framework of constraints These constraints may be intrinsic (e.g., relatedness, sex) or extrinsic (e.g., resource distribution, predation risk) (Alexander 1974). Typically cited benefits of group living include reduced predation threat, improved foraging and mate choice; costs include competition, interference, increased predation and increased parasite burden (Krause & Ruxton 2002). Social structure is not only influenced by intrinsic and environmental factors but can, in turn, affect these in a feedback loop (Geist 1974, Crook et al. 1976, Rubenstein & Rubenstein 2013). Therefore, social structure is a core element of population biology (Wilson 1975) affecting such factors as fitness (McDonald 2007), gene flow (Altman et al. 1996), information flow (Mann et al. 2012, Allen et al. 2013) and disease transmission (Altizer et al. 2003, Cross et al. 2004, Drewe 2010).

In social units comprised of relatives, kin selection should play a role in determining cooperation among group members (Hamilton 1964). In groups comprised of non-kin, direct fitness benefits are expected to contribute to the promotion and maintenance of the social grouping, for example through cooperative hunting in social apex predators such as some carnivores (e.g., Creel & McDonald 1995, Smith et al. 2012). If social organization is indeed closely linked to group hunting, we may expect social organization to be associated with variation in the exploited resource, such as for the differential social organization and dynamics of *resident* and *transient* killer whales in the Northeast Pacific (see Hoelzel 1993; Ford et al. 1998; Baird & Whitehead 2000), possibly related to the level and type of cooperation required (see Hoelzel 1991, 1993). However, variation may also arise because the resource more generally affects the costs and benefits of sociality (Creel & McDonald 1995), and temporal changes in prey resource may affect group size and coherence (Aureli et al. 2008).

Killer whales *Orcinus orca* are an interesting case study: these large-brained, large-bodied dolphins are among the most widely distributed non-human animals. They are apex predators in the marine environment. Numerous studies (reviewed in de Bruyn et al. 2013) have shown that populations – some sympatric – differ in key aspects: morphology, genetics, diet, movement, vocal behavior and social structure. This has led to the recognition of various 'ecotypes', and some authors propose separate or incipient species (e.g., LeDuc et al. 2008, Morin et al. 2010). Other authors point out evidence for ongoing gene flow and shared histories among ecotypes (e.g. Hoelzel et al. 2007, Pilot et al. 2010, Moura et al. 2014). Three well-studied ecotypes occur in the Northeast Pacific: *resident* killer whales feed primarily on salmonid fishes while *offshores* may prey on a broader range of fish species (Krahn et al. 2007), and *transient* killer whales specialize on marine mammals (Ford et al. 1998). Long-term studies of *resident* killer whales have revealed a multi-level social structure with matrilineal units at the core and natal philopatry to these units. Matrilines typically consist of 2-4 maternally related generations and permanent dispersal among communities or populations for identified individuals has not been recorded for either sex. Genetic data indicate ongoing gene flow among matrilines, likely through mating during temporary interactions (Hoelzel et al. 2007, Pilot et al. 2010). Matrilines associate dynamically in larger groups ('pods') of 3-49 individuals (mean = 12.3) and sometimes in multi-pod associations (Bigg et al. 1990, Parsons et al. 2009). In comparison, the social organization of *transient* killer whales is poorly known, but social groups are smaller (1 to ~10 individuals) and seem less stable (Ford & Ellis 1999, Baird & Whitehead 2000). *Offshores* are even less known, but tend to be found in stable mixed-sex groups that are larger than *resident* pods (Dahlheim et al. 2008).

It has been suggested that apparent differences in group size and stability are linked to dietary differences (Hoelzel 1991, 1993, Baird & Dill 1996, Baird & Whitehead 2000). The small number of quantitative descriptions of social structure outside the Northeast Pacific have shown variation in group sizes which seems consistent with this idea. A comparison of two North Atlantic populations (Iceland and Scotland) with common ancestry but contrasting diets (fish and seals, respectively) showed that the seal-eating killer whales had smaller group sizes (mean ± SD = 5.8 ± 3.0) than the fish-eating killer whales (mean ± SD = 14.8 ± 12.0) (Beck et al. 2011).

Quantitative studies of social structure in this cosmopolitan and diverse apex predator may contribute to elucidating the environmental and evolutionary factors influencing sociality in mammals (e.g., Chapman & Rothman 2009). Specifically, killer whales can be used to address the relative roles of inclusive fitness benefits (i.e., kin selection) and direct benefits of cooperative foraging as drivers of sociality in predators. Studies from the southern hemisphere are particularly important as these populations are likely ancestral (Moura et al. 2014, 2015). Given the few studies of social organization in mammal-eating killer whales, we aimed to contribute quantitative information from such a population of killer whales at Marion Island, Southern Ocean. Further, we test the hypothesis that aspects of the social structure among these mammal-eating killer whales are similar to those seen for mammal-eating populations elsewhere. The implication would be that foraging behavior is an important driver of social structure in killer whales, possibly based on competing factors associated with cooperative hunting and energetic constraints. We also test the hypothesis that the strongest associations will be among close kin, which would be consistent with sociality being promoted by kin selection, without proving that this was the case.

METHODS

Ethics clearance

Biopsy sampling was approved by the University of Pretoria's Ethics Committee (EC023-10) and the Prince Edward Islands Management Committee (PEIMC 17/12, 1/2013 and 1/2014).

Marion Island killer whales

Marion Island (46°54′ S, 37°45′ E) and neighboring Prince Edward Island are a pair of small (296 km^2 and 45 km^2, respectively) islands in the Indian sector of the Southern Ocean. The nearest landfall is the Crozet archipelago, ~950 km to the east, and South Africa lies some 1 800 km to the north east. The islands lie in the path of the Antarctic Circumpolar Current, in the Antarctic Polar Frontal Zone, and are characterized by a highly dynamic marine environment (Ansorge & Lutjeharms 2002, Durgadoo et al. 2010). Three seal species and four penguin species breed at the islands and these are hunted by a small (57 individuals at the time of data collection for this study) population of killer whales (Reisinger et al. 2011a). Killer whales may occur at any time of year, but are most abundant during September-December (coinciding with the influx of breeding and molting seals and penguins) and April-May (Reisinger et al. 2011b).

Individual identification

We collected photographic identification data on killer whales from shore at Marion Island from April 2006 to April 2013. Photographs were taken during ‘dedicated observation sessions’ – where an observer would wait for killer whales at a location for a predetermined length of time (usually 3-10 hours) – as well as opportunistic sightings during other field work. When a group was sighted, the observer would estimate the group size and its age-sex composition and attempt to photograph the dorsal fin of each animal in the group, irrespective of the animal’s distinctiveness. The observer continued to take photographs until the group was out of photographic range, irrespective of whether all animals were photographed. Individuals were identified based on natural markings of their dorsal fin and saddle patch – mainly the pattern of nicks, notches and mutilations along the trailing edge of the dorsal fin (Bigg et al. 1987). These were compared to an existing photographic identification catalogue (Tosh et al. 2008, Reisinger et al. 2011a). All photographs were carefully examined and assigned a quality score from 1 (unusable) to 5 (excellent) based on the size of the dorsal fin in the photograph, focus, lighting, exposure, the angle of the dorsal fin to the photographer and the proportion of the dorsal fin obscured by water. Only photographs scoring ≥3 were considered for further analyses (Reisinger et al. 2011a).

Social analyses

Social analyses were performed using SOCPROG 2.6 (Whitehead 2015), run in MATLAB R2015a (The MathWorks, Inc.), and the packages asnipe (Farine 2013) and igraph (Csardi & Nepusz 2006) in R (R Core Team 2015). For these analyses, the initial data format was an individual identification matrix where each row represented a sighting of a group (defined below) and each column an individual, taking a value of 1 if a given individual was photographically identified in a given group and 0 if not. To quantify the proportion of time a dyad spent together we calculated the half-weight association index (HWI) between each pair of individuals (Cairns & Schwager 1987, Whitehead 2008a). This index reduces the bias introduced when not all associates of an individual are identified in a sampling period. We considered individuals associated when they were photographically identified in the same group and we defined a group as individuals within visual range of the observer (usually within 300 meters of each other), moving in the same direction in the same behavioral state (foraging, travelling, resting or socializing, e.g., Ford 1989). In practice, groups were clearly identifiable because sightings were spatio-temporally well defined. Our sampling method involves taking the 'gambit of the group' (Whitehead & Dufault 1999), and assuming that all individuals which occur in a group together are associated. However, this does not necessarily imply that associated animals are socially interacting, and the nature of any social interactions among associated animals is unknown. We defined sampling periods as calendar days, meaning that we assumed animals were associated the entire day if they were observed together on that day. When an individual was sighted in two or more groups during a sampling period it was included in the two or more different groups.

We measured social differentiation (*S*): the variability (measured as CV) of the 'true' (but unobserved) association indices which are approximated using maximum likelihood (Whitehead 2008a, 2008b, 2009) (Supplementary Material). Values close to 0 indicate homogenous relationships within the population while values near or greater than 1 indicate highly varied relationships (Whitehead 2008a). To determine the accuracy of the association indices we calculated the correlation coefficient, *r*, between the maximum likelihood approximation of the 'true', but unobserved association indices (as for *S*) and the observed (measured) association indices (Whitehead 2008a, 2008b, 2009) (Supplementary Material).

To test the null hypothesis that individuals associate at random, we permuted the observations to produce a set of association matrices which can be compared to the real matrix (Manly 1995, Bejder et al. 1998, Whitehead et al. 2005). During permutation group sizes and individual identification frequencies were preserved by swapping pairs of individuals between groups. To control for movement of animals in and out of the study area, but allow enough data for permutations, permutations were constrained within weeks (Bejder et al. 1998). Test statistics for non-random associations are shown in Table 1. We performed 10,000 permutations with 100 trials per permutation and *P* values were calculated as the proportion of times that the test statistics of the permuted data were more extreme than the test statistics of the real data (Whitehead et al. 2005).

We used Newman's (2006) eigenvector-based algorithm for maximizing modularity (*Q*) to detect social modules within the association network. Any modules detected represent densely connected subgroups within the association network and thus correspond to groups of killer whales that are more highly associated with each other than with other killer whales in the population. We compared the observed *Q* value to a distribution of values from 10,000 permutations of the observed data, as described above.

We statistically compared HWIs within versus between social units using a Mantel matrix correlation test (10,000 permutations) based on Spearman's rank correlation (R_M), performed using the vegan package in R (Oksanen et al. 2013). In this test the HWI matrix was compared to a binary matrix

Table 1

Permutation tests for non-random associations among killer whales at Marion Island. Test statistics according to Whitehead (2008a). HWI – half-weight association index. *P* values are calculated as the proportion of times that the permuted test statistics are more extreme than the real test statistic.

Test statistic	Results		
	Real value	Mean of permuted values	*P* value
CV of HWI	3.03	1.61	0.000
SD of HWI	0.13	0.06	0.000
SD of nonzero HWI	0.18	0.06	0.000

where 1 was assigned to a pair of individuals in the same social module, and 0 to a pair of individuals in different social modules (Whitehead 2008a).

To investigate the persistence of associations over time we used standardized lagged association rates (SLARs) (Whitehead 1995). The SLAR is the probability that, given individuals *a* and *b* are associated at some time, a randomly chosen associate of *a* after some time lag will be *b*. We plotted SLAR, as well as the standardized null association rate (the association rate if associations were random), against time lag. We also calculated SLARs for each social module (identified through modularity as described above) to assess the persistence of these social modules over time. Thus, we assessed whether our social modules may correspond with social groupings which are consistently associated over years (e.g., Christal et al. 1998).

Genetic relatedness

We calculated genetic relatedness and estimated parentage among 20 individuals for which we had obtained tissue biopsy samples (sampling methods in Reisinger et al. 2014). DNA extraction was performed using a phenol/ chloroform DNA extraction method (after Hoelzel 1992). Extracted DNA was amplified at 12 microsatellite loci in two separate multiplex PCR procedures, as shown in Supplementary Table S1. All multiplex amplification of microsatellites was performed using a QIAGEN™ Multiplex PCR kit. PCR conditions for multiplex set B were as follows: denaturation of 15 minutes at 95°C; 40 repeat cycles of denature (94°C for 30s), annealing (57°C for 90s) and elongation (72°C for 60s). After the 40 cycles an additional annealing step of 57°C for 90s was added followed by an elongation step of 60°C for 30 minutes. For multiplex set G the PCR conditions differed from set B with only the annealing temperature changing to 50°C. Samples were genotyped on an Applied

Biosystems 3730 ABI DNA Analyzer with size standard ROX500. PCR sizes were visualized on chromatograms produced in GENEIOUS 7.0.5 (Biomatters Ltd.).

The software MICROCHECKER (Van Oosterhout et al. 2004) was used to test for null alleles and other genotyping errors. Hardy Weinberg Equilibrium was assessed using ARLEQUIN 3.5.1.3 (Excoffier et al. 2005). The software KINGROUP v2 (Konovalov et al. 2004) was used to estimate pairwise relatedness values among individuals (*R*; Queller & Goodnight 1989) for Marion Island as a single population (using the pool of all sampled individuals to define allele frequencies). Briefly, *R* is estimated from genotype similarity between two individuals implying common ancestry ('identity by descent'; Blouin 2003); high values indicate highly related individuals, while low values indicate the opposite. Because *R* is related to the population mean allele frequencies, it may take negative values (Queller & Goodnight 1989). The Type II error rate (false discovery rate) was assessed, and the full sibship reconstruction method was implemented to identify clusters of individuals related as parent–offspring pairs, full-siblings, half-siblings or cousins.

We tested the relationship between HWI and genetic relatedness by comparing the rank-based matrix correlation score (R_M) for the observed data to a null distribution of 10,000 correlation scores. Each of these matrix correlations was based on one of the 10,000 permuted data sets produced as described above.

Based on the genotype data we assigned likely parentage using the software CERVUS 3.0.7 (Kalinowski et al. 2007), with all individuals as potential offspring, all adult females as potential mothers and all adult males as potential fathers. An individual was accepted as a likely parent with either a strict confidence level of 95% or a relaxed confidence level of 80%. Relationships which were impossible due to the age and sex of the individuals involved were further excluded. CERVUS was deemed most appropriate given its emphasis on exclusion and categorical allocation, since we had little information on potential sibships and a small sample size (see Jones et al. 2010 for a comparative review of available programs). To provide a second method to help confirm assignments we also used the software COLONY 2.0.6.2 (Jones & Wang 2010), though the emphasis of this program is sibling reconstructions. We based our COLONY analyses on the same assumptions as for the CERVUS runs, using an estimated genotyping error of 0.005 (probability of an allele dropping out) and including all individuals as potential offspring.

RESULTS

We conducted 975 dedicated observation sessions totaling 5 502 'search' hours. We observed killer whales 2 932 times. We took photographs during 1 611 sightings and identified individuals from 1 333 of these sightings. Of these, 1 001 sightings had good quality identifications (photographs with quality scores ≥3). These sightings were made on 481 days, with a mean ± SD of 2.1 ± 1.9 groups (range = 1-14) sighted on these days. We made 2 208 individual identifications from good quality photographs. We identified 57 individuals, including 8 calves born to known individuals during the study. We restricted most analyses to 39 individuals seen on >4 occasions (2 170 individual identifications on 473 days), however, we calculated SLAR using all individuals. The population showed strong social differentiation ($S = 1.353$; SE = 0.019) and the estimated association indices were a useful representation of the true association indices ($r = 0.50$; SE = 0.017). To achieve a 'good' representation ($r = 0.80$), analyses would have to be restricted to animals seen >80 occasions, leaving only 6 individuals. For data where $S^2 \times H > 5$, (where H is the mean number of identifications per individual) the null hypothesis of no preferred or avoided relationships (Table 1) can be confidently accepted or rejected at $\alpha = 0.005$ (Whitehead 2008b). In our data $H = 92.56$, and $S^2 \times H = 169.44$; thus we had sufficient power to test this hypothesis.

We restricted group size analyses to 2 821 sightings when group size was estimated to the individual. Group sizes ranged from 1-16 individuals, with small groups (<5 individuals) most common and a modal group size of 3 individuals (mean ± SD = 2.9 ± 1.4) (Figure 1). Permutation tests showed significant support for non-random associations (Table 1). All individuals were connected in a single network component.

We detected 8 social modules within the network (Figure 2; Supplementary Table S2), an arrangement with modularity significantly greater than the null distribution ($Q_{\text{observed}} = 0.66$, mean $Q_{\text{permuted}} = 0.29$; $P = 0.000$). The modules contained 3-10 individuals each, with a modal size of 3 individuals. Average within-module HWIs were >0.58 in 3 modules, but were low in Modules A, E and F (Figure 3). All modules other than Module D contained at least 2 adult females, and all modules other than Modules C and G contained at least one calf or subadult (Supplementary Table S2).

In the SLAR graph among all individuals (Figure 4a), association rate declined strongly between ~500 and ~747 days, and then more slowly to ~1,805 days. Association rate always remained above the null association rate. When we calculated SLAR within each social module (Figure 4b), association rate remained almost constant throughout the study, indicating that associations within these modules

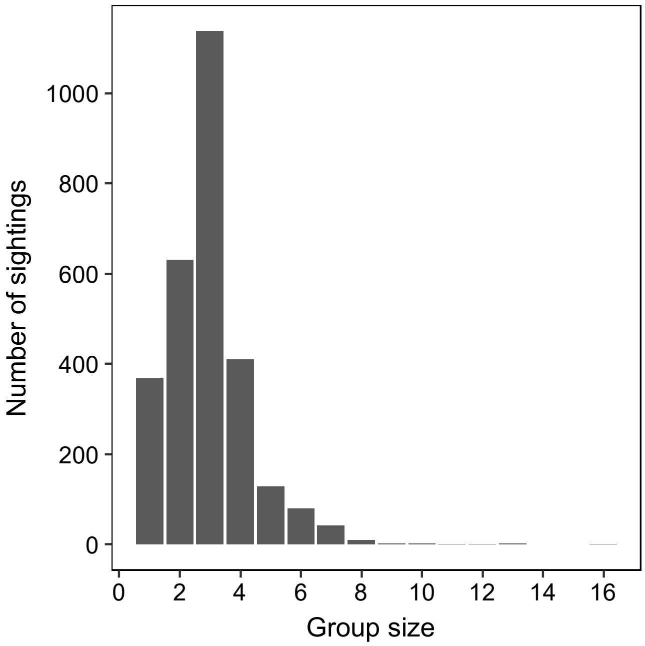

Figure 1

Group size frequency distribution of killer whales at Marion Island.

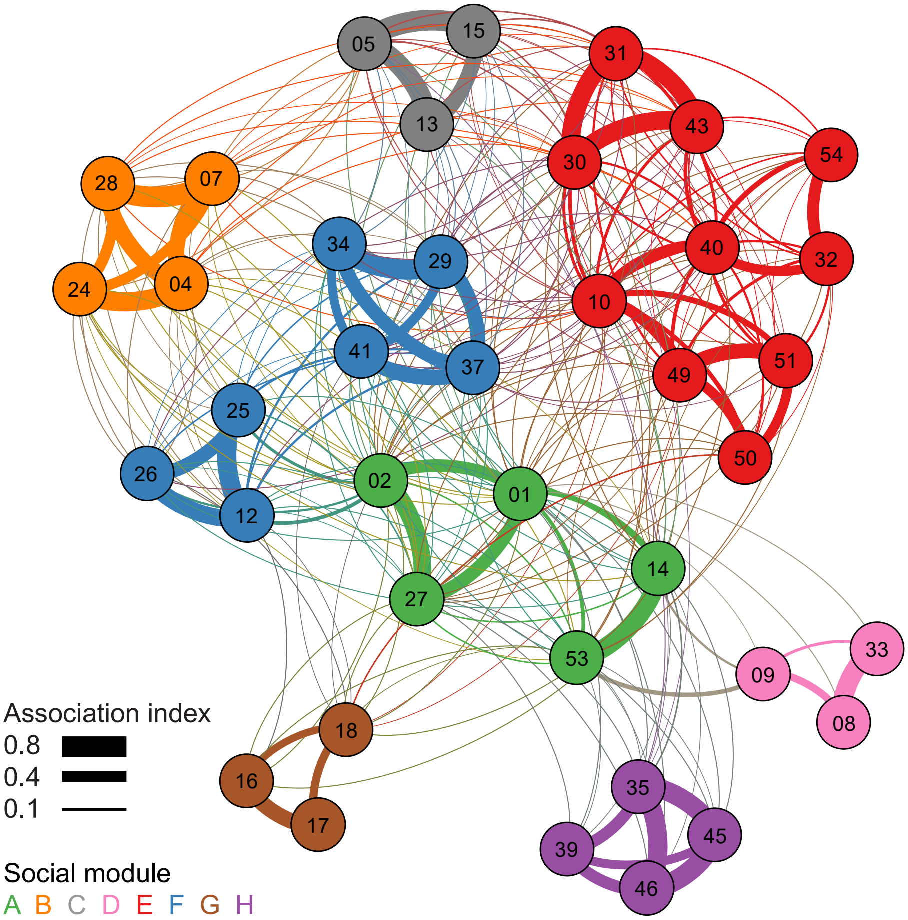

Figure 2

Network graph showing the associations between killer whales at Marion Island. Individuals are represented by nodes (colored circles) and associations by edges (lines) between nodes. Colors represent social modules and edges are weighted by the half-weight association index (HWI). The 'M0' prefix has been omitted from individual labels (e.g., individual M001 is labelled '01'). The graph is laid out using the ForceAtlas2 algorithm (Jacomy et al. 2014) in GEPHI (Bastian et al. 2009).

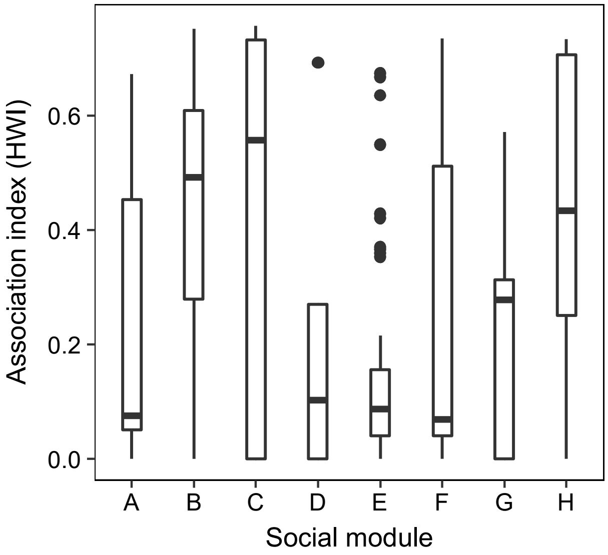

Figure 3

Within-social module association index values of killer whales at Marion Island.

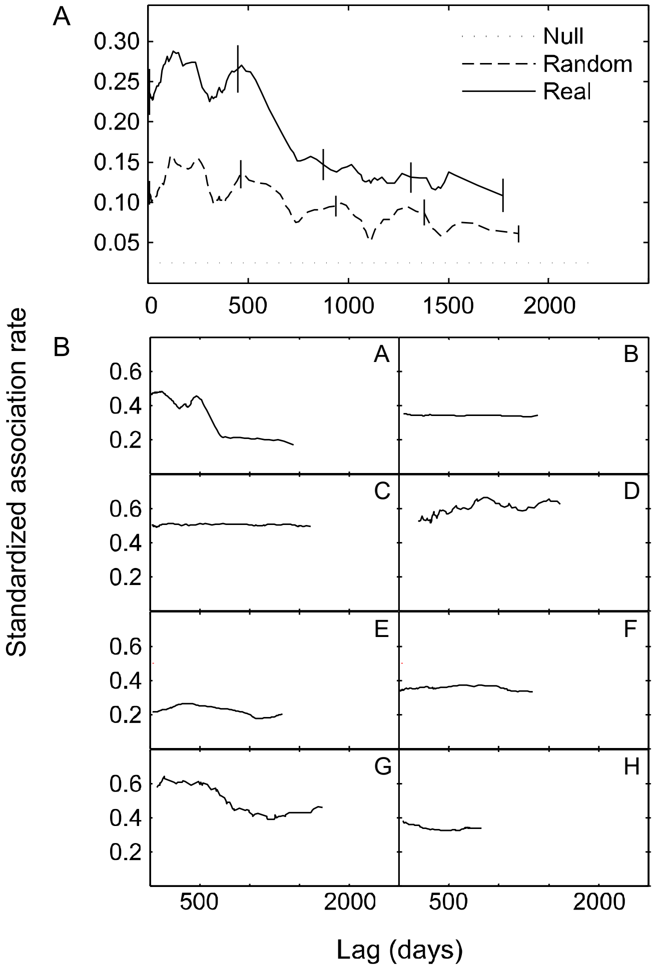

Figure 4

Standardized lagged association rates (SLAR) of killer whales at Marion Island among all individuals (A) and only among individuals in the same social module (B). The SLAR is the probability that, given individuals *a* and *b* are associated at some time, a randomly chosen associate of *a* after some time lag will be *b*. In B, only pairs of individuals in the same social module are considered in the calculation. Standard error (vertical bars) was estimated using a jackknife procedure (Whitehead 2007).

persisted for years. Modules A and G were exceptions; in Module A, association rate fell dramatically after ~500 days, but when we split this module into two sub-modules (a split which could be justified from the hierarchical cluster analysis – see Figure 6a) each sub-module had a stable association rate over the study period (Supplementary Figure S1).

Genetic relatedness

Association and genetic relatedness were not significantly correlated ($R_{\mathrm{M\ observed}} = 0.05$, mean $R_{\mathrm{M\ permuted}} \pm \mathrm{SD} = 0.04 \pm 0.01$; $P = 0.324$). High association index values occurred in dyads with high as well as low relatedness: four dyads had relatedness values below the population mean (i.e., negative relatedness) but had HWIs >0.5 (Figure 5). Within-social module relatedness (Supplementary Table S3) was significantly higher than between module relatedness (mean $R_{\mathrm{within}} \pm \mathrm{SD} = 0.12 \pm 0.34$, range = -0.54 – 0.84, $N = 21$; mean $R_{\mathrm{between}} \pm \mathrm{SD} = -0.05 \pm 0.24$, range = -0.62 – 0.57, $N = 169$; Mantel test based on Spearman's rank correlation: $R_{\mathrm{M}} = 0.19$, $P = 0.006$). A further assessment of the correlation between association and relatedness can be obtained by comparing an average linkage dendrogram constructed using relatedness values, to the dendrogram of HWIs (Figure 6b). The cophenetic correlation coefficient (CCC) between the two dendgrograms (which may range from -1 – 1) was -0.07, indicating that the two dendrograms are statistically different. This value was not significantly different from the expectation for the permuted data (mean $\mathrm{CCC}_{\mathrm{permuted}} \pm \mathrm{SD} = -0.06 \pm 0.02$; $P = 0.85$), consistent with the non-significant R_{M} correlation. The pattern of relatedness within and among age and sex classes was inconsistent, but the number of comparisons is sometimes small (Supplementary Table S3). The pairwise matrix of R-values and information on the significance of tests against a model for full siblings is shown in Supplementary Table S4.

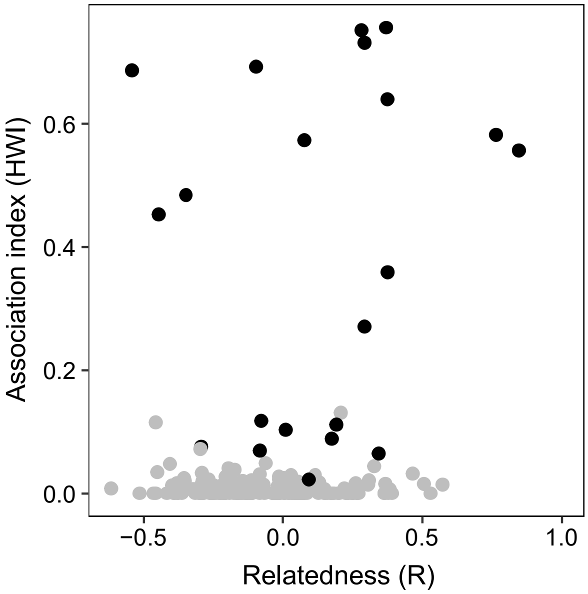

Figure 5

Association index values among 20 killer whales at Marion Island, plotted against their genetic relatedness (*R*). Within-social module values are shown in black, between-social unit values are shown in grey.

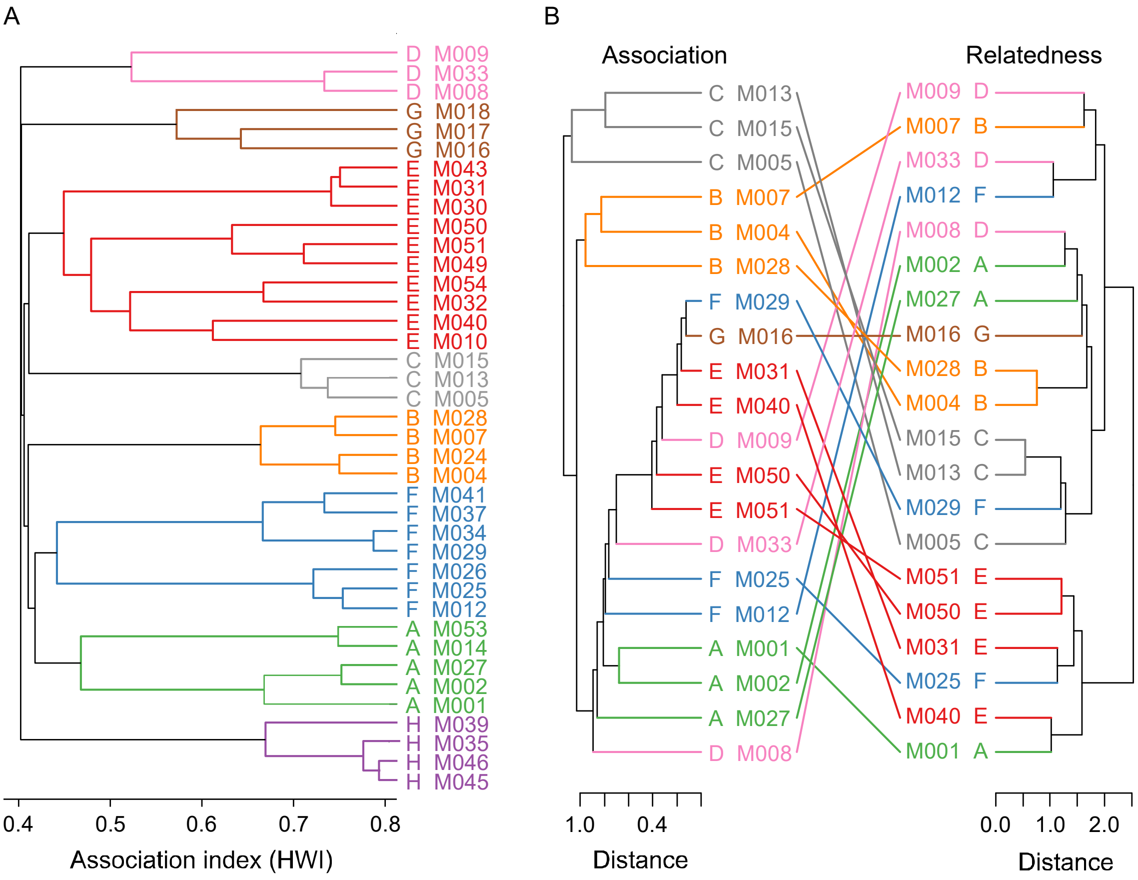

Figure 6

Dendrograms illustrating the association and genetic relatedness among killer whales at Marion Island. A shows associations among 39 killer whales and B shows a ‘tanglegram’ (Galili 2015) comparing dendrograms of association and genetic relatedness among 20 killer whales; in the tanglegram, lines connect the same individuals in the dendrograms being compared. Colours and letters indicate social modules (following Figure 2).

We identified 18 potential offspring-parent pairs, and 6 offspring-mother-father triads, using CERVUS, some of which were supported by the analysis in COLONY (Supplementary Table S5). In only two of these cases were the parents from the same social module, and this was only supported by CERVUS (M004-M007 in Module B and M005-M013 in Module C). There were only two cases where an individual was potentially fathered by an individual in the same social module (CERVUS only, M007-M028 in Module B and M005-M015 in Module C). In five cases (two in COLONY) the offspring and candidate mother were still in the same social module (Supplementary Table S5).

DISCUSSION

Our results show that Marion Island killer whales have a social organization characterized by small social modules of mixed age-sex class composition, which are stable over years but have a degree of fluidity (i.e., fission-fusion) over shorter times. Ten out of 19 pairwise comparisons within social modules suggested close kinship ($R > 0.2$), distributed among all but one of the social modules. At the same time, all but one social module included apparent non-relatives, indicating social dispersal of individuals to join other modules or form new ones. Based on the pattern of putative parent-offspring pairs, mating may be largely between social modules. Aspects of the social organization of Marion Island killer whales appear more similar to those of Northeast Pacific *transient* killer whales than Northeast Pacific *resident* killer whales. However, it is not a perfect match for either model.

There was strong social differentiation among individuals, with clearly preferred long-term associations between individuals. Association rates, however, varied even within social modules. Direct comparisons with other studies are problematic because, in addition to the various association indices used, different sampling methods will affect values of the chosen association index values. Moreover, studies of social organization in Northeast Pacific killer whales have largely been based on genealogies constructed over decades of observation (e.g., Bigg et al. 1990, Ford & Ellis 1999, Ford et al. 2000), rather than quantitative analysis of association patterns (as in our study). However, the group size distribution, general association patterns and social module dynamics we observed seem more consistent with the little that is known of social organization in *transient* killer whales (Ford & Ellis 1999, Baird & Whitehead 2000) than that in *residents* (Bigg et al. 1990, Ford et al. 2000, Parsons et al. 2009).

When comparing the SLAR within modules with that among all individuals it is clear that there is indeed some flexibility or fluidity to association patterns within a framework of stable social modules. Flexible associations may allow individuals or sub-groups to maintain social bonds while optimizing the benefits of grouping, by leaving or joining groups depending on activity or resource distribution (Aureli et al. 2008). This is exemplified by the fission-fusion dynamics of several primates, delphinids, social carnivores and bats (e.g., Nishida 1968, Smolker et al. 1992, Smith et al. 2008, Kerth et al. 2011). Some of the factors which promote fission-fusion in other species (see Gowans et al. 2008) may promote some social flexibility in this population of killer whales. The fluidity we observed may result from: aggregation of individuals at a highly localized resource center; temporary associations for social reasons (alloparental care and mating, discussed below); associations among social modules (a multi-level social structure – see below); or necessity or benefits of cooperative foraging (discussed below).

There is an extensive literature on the ecological determinants of group size in social predators. Caraco and Wolf (1975) showed that group size in prides of foraging lions *Panthera leo* varied with prey in a way that maximized individual fitness. Associations between prey type and group size have also been found for wolves *Canis lupus* (Nudds 1978, MacNulty et al. 2014) and delphinid cetaceans (Gygax 2002). The small group sizes we observed are comparable to those in *transient* killer whales (Marion mean = 2.9, *transient* mean = 4.2 in Baird & Dill 1996). Baird and Dill (1996) proposed that, for *transient* killer whales hunting seals, a group size of three animals maximized per capita energy intake and suggested that this was the primary driver of small group sizes in that population (the ecological constraints hypothesis, Chapman et al. 1995). Hoelzel (1991) found that the number of individuals actively hunting was smaller than the number in the group for killer whales hunting marine mammals in Argentina (consistent with 16 other studies reviewed in that paper), and that it was common for the hunters to provision other members of the group. Group sizes larger than the predicted optimum are frequently observed in other species (Clark & Mangel 1986, Krause & Ruxton 2002) and for mammal-eating killer whales (Hoelzel 1991, Baird & Dill 1996). For killer whales it has been suggested that larger groups may be more likely to be detected by their prey (Baird & Dill 1996), while factors such as kin selection, alloparental care and cooperative foraging (e.g., Hoelzel 1993) could increase group size. Lions, for example, have group sizes larger than that predicted to maximize energy intake and it has been suggested that factors such as inclusive fitness (i.e., kin selection), minimizing food intake variance, cooperative cub defense, group territoriality and female reproductive patterns cause this (Caraco & Wolf 1975, Clark 1987, Packer et al. 1990). Alternatively, optimal group sizes could be dynamic provided that individual fitness remains constant (Sibly 1983, although cf. Giraldeau & Gillis 1985).

Social units and organization

Our use of a community detection algorithm allowed us to define social modules without making any assumptions about their existence or the expected association patterns within them (cf. 'pods' comprised of individuals spending >50% of their time together; Bigg et al. 1990). However, this also complicates comparisons as stated above. The size of our social modules were consistent with the size of marine-mammal eating killer whale social units elsewhere, though Module F (7 individuals) and Module E (10 individuals) were at the high end of the range compared to Northeast Pacific *transient* social units (Ford & Ellis 1999). However, given the low HWI between certain individuals in these two modules (Figure 3), the modules would only rarely occur at full size. This would agree with the observed group size distribution (Figure 1), where groups >8 are rare. Such an arrangement could be analogous to the multi-level social organization of, for example, African elephants *Loxodonta africana* (Wittemyer et al. 2005), geladas *Theropithecus gelada* (Snyder-Mackler et al. 2012) and *resident* killer whales (Bigg et al. 1990). However, we could not detect any statistically significant multi-level structure using a knot analysis (not shown) (Wittemyer et al. 2005, Beck et al. 2011).

Contrary to the expectation for a strongly matrilineal society, genetic relatedness was not significantly correlated with association. While there are errors associated with the estimates of association as well as relatedness, and the latter is due in part to the relatively low power provided by 12 microsatellite DNA loci, the observed pattern (Figure 5) indicates that the general lack of correlation is not solely due to low power or imprecision. There was a great deal of variation and four dyads spent a large proportion of time together (HWI > 0.5), but had below-mean relatedness. Two of these dyads may be explained by changing social module membership (M001-M002 and M001-M027) and it is possible that the other dyads (M008-M033 and M012-M025) result from similar shifts in association prior to the study period. Only a small number of parentage assignments were supported by both analyses run (likely due to both low power in the method applied by COLONY, and false positives in the CERVUS analysis), but these also indicated movement out of natal social groups (Table S5). The pattern is in striking contrast to parentage results from *resident* pods in the eastern Northeast Pacific, where 17 of 18 parental assignments were within the same pod (Ford et al. 2011). Similar long-term associations among non-kin have been seen in another highly social cetacean, the sperm whale *Physeter macrocephalus* (Ortega-Ortiz et al. 2012). In general, the pattern of relatedness and the stable as well as dynamic associations among individuals we describe for Marion Island killer whales bears some resemblance to the pattern in some sperm whale populations (Christal et al. 1998, Mesnick et al. 2003). Non-kin associations suggest that direct fitness benefits (such as cooperative foraging and alloparental care) are important, though it doesn't exclude a role for inclusive fitness during the evolution of sociality.

This pattern of within-module relatedness (social units comprising related as well as unrelated individuals) is very similar to that of female bottlenose dolphins *Tursiops aduncus* – a species showing fission-fusion dynamics – where kinship correlates with association, but is not a prerequisite for group membership (Möller et al. 2006). This is also the case in other species such as hyenas *Crocuta crocuta* (Van Horn et al. 2004), wolves (Vucetich et al. 2004) and chimpanzees *Pan troglodytes* (Lukas et al. 2005), where individuals in cooperative groups are not necessarily related, indicating direct benefits.

In the study by Pilot et al. (2009) the mean within-pod kinship values were 0.127 (±0.058) for Northeast Pacific *transient* pods and 0.363 (±0.047) for the piscivorous *resident* pods. Our findings for the Marion Island social units are intermediate, and the variance is much higher ($\bar{X} \pm \mathrm{SD} = 0.16 \pm 0.35$). This and other features suggest that social structure at Marion is not a perfect match for either the *resident* or the *transient* model from the Northeast Pacific studies, although the latter is not well-known. Our limited parentage analysis indicates mating largely between social units, though inference is relatively weak when mother-offspring pairs cannot be identified *a priori.* This is consistent with results from Northeast Pacific *resident* killer whales where inferred mating is predominantly between pods (Barrett-Lennard 2000, Pilot et al. 2010, Ford et al. 2011).

Conclusions

Our data indicates long-term associations among killer whales at Marion Island, but social group membership was dynamic, and associations with non-kin were common. We note that the observed pattern of association is more like that seen by marine mammal consumers elsewhere than populations that specialize on fish prey, but distinct from both. Prey choice can determine optimal group size and affect association dynamics in apex predators (e.g. Kraus & Ruxton 2002), suggested earlier for killer whales (e.g. Hoelzel 1991, Baird & Dill 1996). We recommend that further data should be obtained to better understand this relationship, and especially the potential for changes in spatial-temporal resource availability over time to influence group composition and dynamics.

DATA ACCESSIBILITY

Analyses reported in this article can be reproduced using the data provided by Reisinger et al. (2017).

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SUPPLEMENTARY MATERIAL TO:

Kinship and association in a highly social apex predator population, killer whales at Marion Island

Ryan R Reisinger[1,2*], Charlene Beukes (née Janse van Rensburg)[1], A Rus Hoelzel[3], PJ Nico de Bruyn[1]

[1] Mammal Research Institute, Department of Zoology and Entomology, University of Pretoria, South Africa

[2] Department of Zoology, Nelson Mandela Metropolitan University, South Africa

[3] School of Biological and Biomedical Sciences, Durham University, United Kingdom

*ryan.r.reisinger@gmail.com

Supplementary methods

Association index

Social analyses were performed in SOCPROG 2.6 (Whitehead, 2009) run in MATLAB R2015a (The MathWorks, Inc.) and the packages asnipe (Farine 2013) and igraph (Csardi & Nepusz 2006) in R (R Core Team 2015). To quantify the proportion of time a dyad spent together we calculated the half-weight association index (HWI) between each pair of individuals:

$$HWI_{ab} = \frac{x}{x+y_{ab}+\frac{1}{2}(y_a+y_b)} \qquad \text{Equation 1}$$

where x is the number of sampling periods where individuals a and b are associated; y_a the number of sampling periods where only a is identified; y_b the number of sampling periods where only b is identified and y_{ab} the number of sampling periods where a and b are identified, but not associated (Cairns & Schwager 1987, Whitehead 2008a). This index reduces the bias introduced when not all associates of an individual are identified in a sampling period.

Social differentiation

We measured the *social differentiation* (S): the variability (measured as CV) of the 'true', but unobserved association indices (Whitehead 2008a, 2008b, 2009). We estimated these 'true' association indices using a maximum likelihood approximation (Equation 23 in Whitehead 2008a) which is considered to be less biased and more precise than the Poisson method for estimating the 'true' association indices. Values close to 0 indicate homogenous relationships within the population while values near or greater than 1 indicate highly varied relationships (Whitehead 2008a).

Accuracy of association indices

To determine the accuracy of the association indices we calculated *r*: the correlation coefficient between the maximum likelihood estimates of the 'true', but unobserved association indices (as above) and the measured (observed) association indices. This can be expressed in terms of *S*, as above (Whitehead 2008a, 2008b, 2009):

$$r = \frac{S}{\mathrm{CV}(HWI_{ab})} \qquad \text{Equation 2}$$

Supplementary tables

Table S1

Microsatellite loci used for DNA amplification in two separate multiplex PCR procedures.

a) Multiplex Mix B

Primer	Primer sequences	Allele range	Repeat Unit	Fluorescent Dye	Reference
EV37Mn	F: 5'AGCTTGATTTGGAAGTCATGA R: 5'TAGTAGAGCCGTGATAAAGTGC	188-222	$(AC)_{24}$	NED	Valsecchi & Amos 1996
Dde09	F: 5' GAAGATTTTACCCTGCCTGTC R: 5' GATCTGTGCTCCTTAGGGAAA	229–241	$(CTAT)_{10}$	FAM	Coughlan et al. 2006
Dde70	F: 5' ACACCAGCACCTACATTCACA R: 5' TCAGCAGCATTCTAACCAAAC	124–140	$(CA)_{21}$	HEX	Coughlan et al. 2006
Dde65	F: 5' GGTAGTCGTAGGGAAAGGGTA R: 5' AGCAGCCCTAGCAACCTTATA	176–180	$(CTAT)_{13}$	FAM	Coughlan et al. 2006
Dde69	F: 5' TTTCAGTAGTGTGCATGTGTAT R: 5' GAATACCAGAGGGCAAGG	190–206	$(GATA)_{13}$ $(GA)_{21}$	HEX	Coughlan et al., 2006
Dde72	F: 5' TGCTCAACAGATTTCACACTT R: 5' AAGGAAACAAAGTATCTGAGCA	251–279	$(CTAT)_{15}$	HEX	Coughlan et al. 2006
Dde66	F: 5' AACATTGCCAGTGCCTTAGAA R: 5' GTGGAACAGACGCGCATAT	349–373	$(GT)_{19}$	FAM	Coughlan et al. 2006
EV14Pm	F: 5' TAAACATCAAAGCAGACCCC R: 5' CCAGAGCCAAGGTCAAGAG	142-150	$(GT)_{11}$	NED	Valsecchi & Amos 1996

b) Multiplex Mix G

Primer	Primer sequences	Allele range	Repeat Unit	Fluorescent Dye	Ta (°C)	Reference
KWM2a	F: 5' GCTGTGAAAATTAAATGT R 5' CACTGTGGACAAATGTAA	143-157	dinucleo	FAM	47.3	Hoelzel et al. 1998
KWM12a	F: 5' CCATACAATCCAGCAGTC R 5' CACTGCAGAATGATGACC	168-184	dinucleo	NED	56	Hoelzel et al. 1998
TexVet5	F: 5' GATTGTGCAAATGGAGACA R 5' TTGAGATGACTCCTGTGGG	236-260	$(CA)_{24}$	FAM	51	Rooney et al. 1999
AAT44	F 5' CCTGCTCTTCATCCCTCACTAA R 5' CGAAGCACCAAACAAGTCATAGA	98-110	$(AAT)_{12}$	HEX	55	Caldwell et al. 2002

Table S2

Composition of social modules identified based on Newman's (2006) leading eigenvector community detection algorithm. AF – adult female, AM – adult male, C – calf, SA – subadult. Known mother-calf relationships are indicated by "↘" preceding the calf ID.

Social module	Individual	Class
● Module A	M001	AM
	M002	AF
	↘ M027	C
	M014	AF
	M053	SA
● Module B	M004	AF
	↘ M024	C
	M007	AM
	M028	AF
● Module C	M005	AM
	M013	AF
	M015	AF
● Module D	M008	AM
	M009	SA
	M033	AF
● Module E	M010	AM
	M030	AM
	M031	AF
	M032	AF
	M040	SA
	M043	SA
	M049	AF
	M050	SA
	M051	SA
	M054	SA
● Module F	M012	AF
	M025	SA
	M026	AF
	M029	AF
	↘ M034	C
	M037	AF
	M041	SA
● Module G	M016	AM
	M017	AF
	M018	AF
● Module H	M035	AF
	↘ M039	C
	M045	AF

	M046	SA

Table S3

Genetic relatedness (*R*) among killer whale individuals in the same social module, following Figure 2. Letters in parenthesis indicate age sex class of the individual as follows: AF – adult female, AM – adult male, C – calf, SA – subadult.

● Module A	M001 (AM)	M002 (AF)	M027 (C	
M001 (AM)				
M002 (AF)	-0.41			
M027 (C)	-0.34	0.37		
● Module B	M004 (AF)	M007 (AM)	M028 (AF)	
M004 (AF)				
M007 (AM)	0.08			
M028 (AF)	0.76	0.28		
● Module C	M005 (AM)	M013 (AF)	M015 (AF)	
M005 (AM)				
M013 (AF)	0.29			
M015 (AF)	0.37	0.84		
● Module D	M008 (AM)	M009 (SA)	M033 (AF)	
M008 (AM)				
M009 (SA)	0.29			
M033 (AF)	-0.10	0.01		
● Module E	M031 (AF)	M040 (SA)	M050 (SA)	M051 (SA)
M031 (AF)				
M040 (SA)	0.21			
M050 (SA)	0.08	0.17		
M051 (SA)	0.31	-0.08	0.38	
● Module F	M012 (AF)	M025 (SA)		
M012 (AF)				
M025 (SA)	-0.54			

Table S4

Relatedness values derived using KINGROUP with significance shown testing against a model for full sibs.

	M001	**M002**	**M004**	**M005**	**M007**	**M008**	**M009**	**M012**	**M013**	**M015**	**M016**	**M025**	**M027**	**M028**	**M029**	**M031**	**M033**	**M040**	**M050**	**M051**
M001		-0.4464	-0.4508	-0.2831	0.1298	-0.6170	-0.2668	0.0090	-0.2165	-0.1717	-0.1098	0.3812	-0.3483	-0.2009	-0.3519	0.3080	0.0966	0.5722	0.2187	0.0641
M002	-0.4464		0.3667	0.0434	-0.1732	0.5278	0.0447	0.2076	0.0566	0.1494	0.3047	-0.4571	0.3735	0.2607	-0.0834	-0.3982	0.2708	-0.3238	-0.0693	-0.3692
M004	-0.4508	0.3667		0.1017	0.0760	0.2504	0.2296	0.0030	0.0847	0.3762	-0.0370	-0.2057	0.2625	0.7638	0.1149	-0.2299	0.1829	-0.1777	-0.2920	-0.2084
M005	-0.2831	0.0434	0.1017		-0.0811	0.0309	-0.1325	-0.3632	0.2926	0.3699	0.0749	0.0541	0.1753	0.1090	0.3263	-0.4056	-0.2628	-0.1681	-0.2096	-0.2883
M007	0.1298	-0.1732	0.0760	-0.0811		-0.2414	0.0582	-0.0156	0.0540	-0.0224	0.0636	0.0284	-0.2657	0.2805	-0.2905	-0.1127	0.0542	-0.1077	-0.0670	-0.1503
M008	-0.6170	0.5278	0.2504	0.0309	-0.2414		0.2915	-0.2203	0.2641	0.2655	0.1476	-0.2941	0.2276	-0.1430	0.2326	-0.1663	-0.0967	-0.3144	0.0021	0.1600
M009	-0.2668	0.0447	0.2296	-0.1325	0.0582	0.2915		-0.1658	0.0652	-0.0130	0.0717	0.1266	-0.2604	0.0371	-0.0343	-0.0164	0.0091	-0.3917	-0.0110	0.1507
M012	0.0090	0.2076	0.0030	-0.3632	-0.0156	-0.2203	-0.1658		-0.0280	0.0737	0.0215	-0.5425	-0.1959	0.0543	-0.2930	-0.4561	0.3896	-0.1308	-0.1585	-0.5138
M013	-0.2165	0.0566	0.0847	0.2926	0.0540	0.2641	0.0652	-0.0280		0.8446	0.3632	-0.2477	-0.1270	0.0471	0.5048	-0.1832	-0.2297	-0.2254	-0.2574	-0.2512
M015	-0.1717	0.1494	0.3762	0.3699	-0.0224	0.2655	-0.0130	0.0737	0.8446		0.2431	-0.2129	0.0071	0.2016	0.4638	-0.2971	-0.0838	-0.1140	-0.2997	-0.3551
M016	-0.1098	0.3047	-0.0370	0.0749	0.0636	0.1476	0.0717	0.0215	0.3632	0.2431		-0.1139	0.0237	0.0199	-0.1188	-0.2062	0.1707	-0.0300	-0.1125	-0.1309
M025	0.3812	-0.4571	-0.2057	0.0541	0.0284	-0.2941	0.1266	-0.5425	-0.2477	-0.2129	-0.1139		-0.0634	-0.1459	-0.0828	0.3629	-0.2870	0.2249	0.0216	0.1966
M027	-0.3483	0.3735	0.2625	0.1753	-0.2657	0.2276	-0.2604	-0.1959	-0.1270	0.0071	0.0237	-0.0634		0.1116	-0.0134	-0.3506	-0.2999	0.1235	-0.1296	-0.2039
M028	-0.2009	0.2607	0.7638	0.1090	0.2805	-0.1430	0.0371	0.0543	0.0471	0.2016	0.0199	-0.1459	0.1116		-0.0196	-0.2971	0.2520	-0.2476	-0.3760	-0.4166
M029	-0.3519	-0.0834	0.1149	0.3263	-0.2905	0.2326	-0.0343	-0.2930	0.5048	0.4638	-0.1188	-0.0828	-0.0134	-0.0196		-0.1983	-0.3897	-0.3818	-0.4648	-0.0376
M031	0.3080	-0.3982	-0.2299	-0.4056	-0.1127	-0.1663	-0.0164	-0.4561	-0.1832	-0.2971	-0.2062	0.3629	-0.3506	-0.2971	-0.1983		-0.3788	0.1916	0.0924	0.3428
M033	0.0966	0.2708	0.1829	-0.2628	0.0542	-0.0967	0.0091	0.3896	-0.2297	-0.0838	0.1707	-0.2870	-0.2999	0.2520	-0.3897	-0.3788		-0.1926	-0.1597	-0.2860
M040	0.5722	-0.3238	-0.1777	-0.1681	-0.1077	-0.3144	-0.3917	-0.1308	-0.2254	-0.1140	-0.0300	0.2249	0.1235	-0.2476	-0.3818	0.1916	-0.1926		0.1736	-0.0787
M050	0.2187	-0.0693	-0.2920	-0.2096	-0.0670	0.0021	-0.0110	-0.1585	-0.2574	-0.2997	-0.1125	0.0216	-0.1296	-0.3760	-0.4648	0.0924	-0.1597	0.1736		0.3755
M051	0.0641	-0.3692	-0.2084	-0.2883	-0.1503	0.1600	0.1507	-0.5138	-0.2512	-0.3551	-0.1309	0.1966	-0.2039	-0.4166	-0.0376	0.3428	-0.2860	-0.0787	0.3755	

***p*:**

< 0.005

< 0.05

< 0.1

Table S5

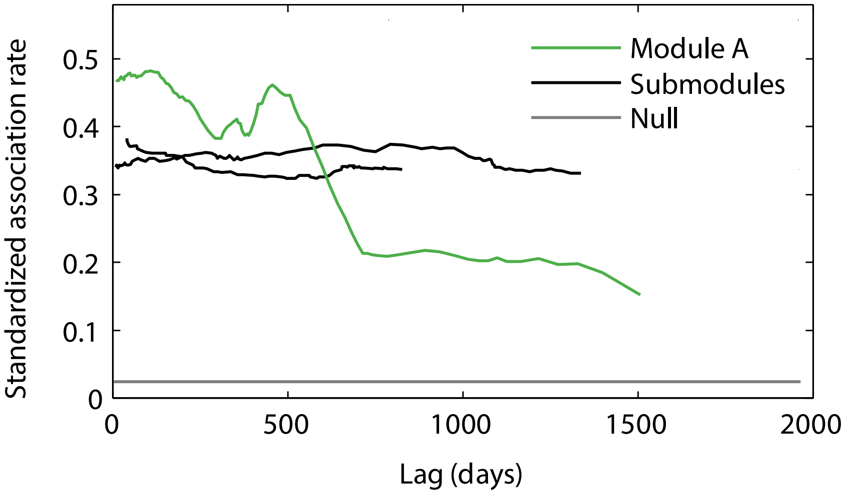
Potential father-mother-offspring triads among killer whales at Marion Island based on CERVUS and COLONY results. All CERVUS results shown had confidence >80% for pairwise relationships, and all triads had confidence >95%. Pairwise relationships with confidence >95% are indicated with an asterisk. In COLONY, only the triad probability is given. Dashes indicate that parentage was not assigned. Colors and letters in brackets indicate the individual's social module, following Figure 2. Bold type indicates relationships where relatedness (*R*) is >0.5 (see Supplementary Table S4).

Offspring	CERVUS		COLONY		
	Candidate mother	Candidate father	Candidate mother	Candidate father	Probability
M001 (A)	M012	M007 (B)*	-	-	-
M002 (A)	-	**M008 (D)***	-	**M008 (D)**	0.99
M004 (B)	M002 (A)	-	-	-	-
M005 (C)	-	M016 (G)*	M029 (F)	-	0.76
M007 (B)	M004 (B)	M001 (A)*	-	-	-
M008 (D)	**M002 (A)***	-	-	-	-
M009 (D)	M004 (B)	-	-	-	-
M013 (C)	**M015 (C)***	M016 (G)*	**M015 (C)**	-	0.83
M015 (C)	**M013 (C)***	M005 (C)*	-	-	-
M016 (F)	M013 (C)	-	-	-	-
M025 (G)	M031 (E)*	M005 (C)*	M031 (E)	M005 (C)	0.88
M027 (A)	M002 (A)*	-	M002 (A)	-	0.97
M028 (B)	**M004 (B)***	M007 (B)*	-	-	-
M029 (F)	**M013 (C)***	-	**M013 (C)**	-	0.76
M031 (E)	-	M001 (A)*	-	-	-
M033 (D)	M012 (F)*	-	-	-	-
M040 (E)	M031 (E)	-	-	-	-
M050 (E)	-	M001 (A)*	-	-	-

Supplementary figure

Figure S1

Standardized lagged association rates (SLAR) of social Module A, as well as two sub-modules defined by splitting Module A (M001-M002-M027 and M014-M053). The SLAR is the probability that, given individuals *a* and *b* are associated at some time, a randomly chosen associate of *a* after some time lag will be *b*.



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