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Indirect detection of genetic dispersal (movement and breeding events) through pedigree analysis of dugong populations in southern Queensland, Australia

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ABSTRACT

Understanding the patterns of movement and breeding within and between wildlife populations is important for the assessment of conservation status of endangered species, the development of conservation management strategies and priorities, and the prediction of population behaviour based on future threats. Methods for determining long term gene flow and dispersal are well researched, but analysis of recent movement is more difficult, typically relying on real-time tracking of individuals using telemetry, or through identification of marked individuals at multiple locations. These methods are limited by the considerable sampling effort required over time periods sufficient to recapture individuals in multiple locations. In contrast, we can infer recent movement from a reconstructed pedigree based on genetic and ancillary biological data, by identifying parent-offspring relationships in which the parent and offspring may be found in different locations. Hence, this method can use a single sampling period to identify movement and possibly associated breeding events over the last one or two generations. This study demonstrates the utility of reconstructed pedigrees in inferring recent movements in a dugongs distributed across a number of spatially distinct foraging locations in southern Queensland, Australia. Dugongs, which are classified as vulnerable to extinction, have long lifespans and protracted breeding cycles and give birth to single offspring at irregular intervals, implying a complex pedigree without distinct generational structure or large sibling groups. A pedigree was constructed for 1002 different dugongs across four locations in southern Queensland: Moreton Bay ($n = 630$), the Great Sandy Straits ($n = 281$), Hervey Bay ($n = 59$) and Shoalwater Bay ($n = 27$) using *PR-genie* software. *PR-genie* is a pedigree reconstruction system designed specifically for complex multigenerational wildlife pedigrees based on genetic identity (microsatellite DNA) and including ancillary biological data (sex and body size-class). Movements of genetically tagged individuals between locations were detected as parent-offspring links found across locations. Approximately 30% of assigned parents had at least one offspring found in a different location, implying recent movement of the parent or offspring. Where multiple individuals in a family are present, parsimonious explanations of movement indicate that male dugongs move between populations more frequently than females. Markedly more movement between locations was found than has been previously possible to detect through repeated direct sampling of individuals or through telemetry.

1. Introduction

The genetic analysis of populations, population structure and genetic dispersal is of significant interest in a wide variety of biological applications. In wildlife populations, genetic parameters are crucial for conservation, as efficient management of any species requires at least a basic understanding of their population dynamics (Hampton et al., 2004). Patterns of genetic diversity within populations and genetic differentiation amongst populations are important for the assessment of the spatial extent of endangered species (Blouin et al., 2010) and development of appropriate conservation strategies (Excoffier and Heckel, 2006).

The F statistics developed independently by Wright (1949) and Malĕcot (1948) provide convenient measures of genetic

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differentiation among and within populations, and have long been used to infer demographic history, estimate movement rates and identify regions of the genome under selection (Holsinger and Weir, 2009). Population structure provides insight into the levels of dispersal and connectivity between wildlife populations, because populations that are genetically distinct have likely had little exchange or interbreeding, whilst populations with little structuring have had significant immigration/emigration and interbreeding. Coalescent methods are an alternative method of determining models for population structure and for the direct estimation of movement rates between populations, such as those implemented in the software Ima2 (Hey and Nielsen, 2007) or Migrate-n (Beerli and Palczewski, 2010). Assignment testing, i.e., the assignment of individuals to populations based on their genetic composition, can provide insight not only into the long-term differentiation between populations, but into movement on an individual level in the short term. Movement can be inferred, for example, where an individual found in one location is assigned genetically to a population found in a different location. A relevant method of assignment testing is via Bayesian clustering, which infers population structure without assuming predefined populations (Chen et al., 2007), with the program STRUCTURE (Pritchard et al., 2000) being the most influential system to implement these techniques (François and Durand, 2010).

Whilst long-term population structure parameters are valuable and assignment testing provides some insight into movements (e.g., Nater et al., 2012), information on recent movements and effects on population structure of wildlife is critical for population management. Such data are used to address significant questions in conservation such as determining immediate causes of fluctuations and particularly declines in population size, and hence distinguishing movement events from mortality events, particularly in relation to anthropogenic or natural disturbance. Typically, short term movement patterns have been understood through direct observation, such as the tracking of individuals using telemetry (e.g., Maxwell et al., 2011), or through identification of individual animals in multiple locations, either by natural discriminatory marks or physical or genetic tags, e.g., Wells et al. (2008). While they can be effective in many situations depending on the question being asked, these methods come at considerable expense, require significant sampling effort over the duration of a study to recapture or re-observe individuals in multiple locations (particularly difficult for cryptic species), can be disrupted by loss or changes in discriminatory markings such as scar patterns, and can only detect those movements that occur during the study. In contrast, the use of genetic methods to detect contemporary movements, i.e., movements occurring within the lifetimes of extant individuals, can overcome some of these challenges, in particular the necessity of observing the same individual in multiple locations, and the limitation to movements that occur within the duration of the study. The use of genetic assignment testing, however, requires sufficiently genetically distinct populations, and may not provide significant insight into the timing of movement events nor indicate if these were accompanied by subsequent breeding and hence gene flow into the new location. Recently, assignment of parent-offspring relationships between individuals has been used to infer contemporary dispersal in wildlife, with Waser and Hadfield (2011) finding similar rates of dispersal though recapture and parentage analysis methods informed by spatial data for banner-tailed kangaroo rats (*Dipodomys spectabilis*). Here, we suggest that constructing a pedigree based primarily on genetic data will provide insight into contemporary movements and breeding events, by identifying individuals observed in different locations to their parents, siblings or offspring. We demonstrate this technique by considering the dugong (*Dugong dugon*) populations of southern Queensland, Australia.

The dugong is a large marine mammal inhabiting tropical and subtropical regions of the western Pacific and Indian oceans, its range covering the territories of over 37 countries (Marsh, 2002). Dugong are classified by the IUCN as vulnerable to extinction (Marsh, 2008), and aerial surveys over the past few decades indicate that significant population declines have occurred throughout their range and that many populations are currently under threat (Marsh et al., 2001a; Marsh and Lawler, 2001c; Sobtzick et al., 2012). Their long lifespans, protracted breeding cycles, and specialised seagrass diets make dugongs vulnerable to human impact, particularly where their habitats are close to large population centres (Marsh, 2002). In particular, dugong populations along the urban coast of southern Queensland, Australia, are found close to developed population centres featuring significant industrial and coastal activity. Modelling of long term trends in dugong bycatch in a government shark control program have indicated that significant declines in dugong populations may have occurred along the entire southern Queensland coast, to approximately 3% of 1960 population levels (Marsh et al., 2001a, 2005). Within south-east Queensland, the majority of dugongs are found in two spatially distinct foraging areas (300 km apart): in Moreton Bay (MB) and the Hervey Bay-Great Sandy Straits (HB-GSS) region, each of which includes designated sanctuary areas. Aerial surveys conducted over the past two decades have indicated short-term fluctuations in the HB-GSS region; a population of 2206 ± 420 in 1988 (Lee Long et al., 1993) declined to 807 ± 151 in 1994 (Marsh et al., 1996) after flood-associated loss of seagrass in 1992 (Preen and Marsh, 1995), and was then documented as 1654 ± 248 in 1999 (Marsh and Lawler, 2001b), 2547 ± 410 in 2005 (Marsh et al., 2006), and 2116 ± 108 in 2011 (Sobtzick et al., 2012), i.e., population estimates varied between 36% and 115% of the 1988 estimate. In MB, population estimates have ranged from 442 ± 69 in 1988 (Preen, 1992), 968 ± 44 in 1995 (Lanyon et al., 2003), 454 ± 41 in 2005 (Marsh et al., 2006) to 883 ± 63 in 2011 (Sobtzick et al., 2012), i.e., population estimates varying up to 219% of the 1988 estimate. However, because survey methodology in MB has not been consistent, population trends are doubtful, likely reflecting changes in survey technique rather than actual changes in population (Marsh, 2002; Lanyon et al., 2003). All estimates are 95% confidence intervals.

In order to appropriately manage threats to the south-east Queensland dugong populations, it is important to understand population connectedness and determine if population fluctuations in the region have been due to local mortality and/or large-scale movements between locations. Telemetry-based studies have previously indicated that dugongs are capable of large-scale movements of up to a maximum observed journey of 560 km by one individual, with a further 14 of 70 tagged individuals making movements of over 100 km (Sheppard et al., 2006). On a larger scale, gene-flow based studies on dugongs suggest significant dispersal between populations Australia-wide, and indicate that population-genetic structure exists on large geographic scales (Blair et al., 2013). Comparisons between population structure suggested by mitochondrial DNA and nuclear DNA indicated that gene-flow has been primarily male-mediated (McDonald, 2006). More recently, population genetic analysis has indicated low but significant population differentiation within southern Queensland (e.g., $F_{ST} = 0.021$ between MB and GSS), and a Bayesian clustering analysis (via STRUCTURE) suggested two clusters, primarily distinguishing MB dugongs from those in the more northern populations (Seddon et al., 2014).

When large-scale movement is likely to be occurring, insight into the extent of movements between foraging areas is required, particularly to determine if these are routine or occur only in response to major disturbances or environmental stressors. In 1992, the combination of a cyclone and flooding events caused sig-

nificant seagrass death in the HB area (Preen and Marsh, 1995), corresponding to an apparent decline in the size of the local dugong populations, as well as to an unusually high number of recovered dugong carcasses, i.e., high mortality. It was suggested that the adjacent MB population may have increased by approximately 100 dugongs at this time (Preen and Marsh, 1995). Such large scale movement has been proposed as an explanation for fluctuations in dugong populations elsewhere, i.e., in Western Australia (Gales et al., 2004) and in the Torres Strait (Marsh et al., 2004). It is difficult to detect trends in abundance in marine mammals because they are typically hard to observe and identify (Marsh, 1995), and Wade (1998) suggests that it is likely easier to detect circumstances which might lead to population decline than to detect the decline itself. Having knowledge of the patterns of movement and breeding between dugong populations along a coastal strip would allow us to better understand the apparent fluctuations in population size that have been observed, and permit forecasting of changes in abundance in the face of future threats, so that appropriate risk-management strategies can be established.

In 2001, a population capture-mark-recapture program was initiated for dugongs in MB, southern Queensland, Australia (Lanyon et al., 2002), with individuals genotyped for identity using a panel of 24 microsatellite markers (Broderick et al., 2007). More than 600 live individuals were sampled over this period (71% of 2011 population estimates; Sobtzick et al. (2012)), providing a large amount of biological and genetic recapture data. This thorough survey of a single population has provided insight into the distribution of individuals in the region (Lanyon et al., 2003, 2005) and to aspects of their life histories (Lanyon et al., 2009a; Burgess et al., 2012a,b). Individuals in other locations in southern Queensland have been sampled for genetic data: approximately 60 live individuals from HB and more than 400 from the GSS. A small number (approx. 30) of dugongs from Shoalwater Bay (SB), a dugong foraging location ~500 km north of HB, has also been sampled and these are included in this study.

Reconstruction of a pedigree for dugong populations is challenging for a number of reasons. Dugongs are long-lived, breed infrequently and have only one offspring at a time, at irregular intervals: as a result, sibling groups are small and not directly observable (Marsh et al., 1984). Dugongs are understood to undertake promiscuous mating, either via scramble promiscuity or lek mating (Marsh and O'Shea, 2012), assumed here to be effectively random. Generational structure is neither clear nor distinct, as once maturity has been reached, offspring are indistinguishable from their parents in terms of age, and thus relative age data is only available for individuals first sampled as calves/juveniles or sub-adults. A pedigree reconstruction system *PR-genie* (Cope et al., 2014) has been developed specifically for difficult circumstances such as these, taking into account genetic and ancillary biological information such as sex and size/maturity class to reconstruct complex, multigenerational pedigrees. The aim of this study was to use these field and genetic data to demonstrate the use of a large reconstructed pedigree to infer contemporary genetic dispersal for wildlife, in this case, the dugong populations of southern Queensland.

2. Methods

2.1. Sample collection

Dugongs in Moreton Bay (MB), Queensland, Australia (27.4° S) were sampled as part of a decade-long ongoing year-round capture-mark-recapture program (Lanyon et al., 2002) that began in 2001, with small numbers of dugongs sampled in 1998–99 as part of a pilot study. Dugongs in the southern and central Great Sandy Straits (GSS, 25.8° S) were sampled on annual trips since 2006, and in the Burrum Heads region of Hervey Bay (HB, 25.2° S) in 2010–2011. HB and GSS have previously been considered a single population (Tikel, 1997; McDonald, 2006; Sobtzick et al., 2012), but in this sampling program they were considered separately so as to increase spatial resolution of populations. Dugongs in Shoalwater Bay (SB, 22.3° S) were sampled in 2007. Prior to most sampling, aerial surveys were performed on the preceding day, flying at an altitude of ~300 m over known dugong aggregation sites and recording the position of observed groups using a GPS. On the day of sampling, boat transects were conducted across locations identified from the air until groups or individual dugongs were encountered, at which time sampling was performed opportunistically. Dugongs of both sexes and all body size classes were sampled. As the program progressed, a greater proportion of the population was sampled as recaptured individuals.

For this project, the primary sampling aim was to genetically tag as many individuals at each location as possible through collection and DNA analysis of skin samples (Lanyon et al., 2002; Broderick et al., 2007). Skin biopsies were taken from the dorsum of each dugong using a hand-scraper, pole-scraper or biopsy punch (Lanyon et al., 2010a). Skin samples were stored in salt-saturated DMSO and frozen at −20 °C until analysed.

Sampling took one of two forms: routine in-water sampling in which dugongs were captured and sampled (Lanyon et al., 2002, 2006), and skin sampling without capture (Lanyon et al., 2010a). Both methods resulted in the collection of dorsal skin samples for genetic analysis, but differed in the available ancillary biological data. In-water sampling after capture has been the primary method of sampling in MB since the inception of the program (Lanyon et al., 2002). Body measurements included body length (snout to fluke notch in a straight line), fluke width, and girths at each of peduncle, anal, umbilical and axillar positions. Sex was visually assessed by an experienced sampler (Lanyon et al., 2009a), faecal samples were taken for steroid hormone analysis, and the presence of secondary sex characteristics (tusks and teats) was recorded (Burgess et al., 2012b). Each dugong was fitted with a unique numerically-coded titanium turtle tag on the trailing edge of the tail fluke, and a 'cookie' notch was clipped in a consistent position on the trailing fluke-edge with a cattle ear notcher to denote a tagged animal and as an additional biopsy sample. Photos of the entire body, but particularly of the fluke (prior to and after physical tagging), were taken. Any distinctive body features such as unusual pigmentation or heavy scarring were recorded. If a dugong was a recaptured individual, the original tag number was recorded and photographed.

Skin sampling without capture was performed when in-water sampling was not possible due to physical, behavioural, ethical or other reasons, and was the primary means of sampling in locations other than MB, i.e., in HB, GSS and SB (Lanyon et al., 2009b, 2010a). In these cases, both genetic identity and sex were determined through molecular analysis of skin, and body length (cm) was estimated visually *in situ* by an experienced observer. When cow-calf pairs were encountered, these were typically both sampled without capture.

The validity of biological data was verified: in-water length measurements determined to be within ±5% for 29 of 30 dugongs also measured out-of-water in a separate study (Lanyon et al., 2010b); sex assigned visually was checked against molecular sexing based on a multiplex PCR assay that amplified the male-specific SRY gene and differentiated ZFX and ZFY gametelogues (McHale et al., 2007). Visual sex discrimination was congruent with molecular sexing in 96% of 454 individuals where both data were available, with mismatches likely due to observer inexperience, occurring during the earlier years of the CMR program (Lanyon

et al., 2009b). Faecal samples were used to determine maturity and reproductive state based on progesterone and testosterone metabolite levels (Burgess et al., 2012a,b), and after comparison with body length measurements, these data were used to classify individuals into body size/ maturity classes: calf (<220 cm), juvenile/ sub-adult (between 220 cm and 250 cm), and adult ($\geqslant$250 cm).

2.2. Identification of individual dugongs

A critical requirement of this study was the unambiguous and consistent identification of individual dugongs and the subsequent matching of recaptured individuals through genetic and/or physical tags. It was recognised that other ancillary biological data, i.e., body size and maturity, would change through time as individuals grew and reached maturity respectively, and that reproductive state would vary seasonally (Burgess et al., 2013). The process of identifying recaptured individuals consisted of initial genetic analysis of tissue samples, matching these genotypes to previously captured individuals to determine likely recaptures, and then validation of these matches using all available biological data.

Tissue samples were genetically analysed using a suite of 24 dugong-specific microsatellite loci developed to identify individual dugongs: this panel has a Probability of Identity (Waits et al., 2001) $P_{ID} = 1.6 \times 10^{-15}$ in this dataset (Broderick et al., 2007). Observed average heterozygosity was in the range 0.48–0.52, and allele richness in the range 4.3–4.5 (Seddon et al., 2014). Full detail as to the microsatellite diversity and marker utility of this dataset are available in Seddon et al. (2014). DNA extraction from skin samples was performed using a standard salting-out method after overnight digestion with Proteinase K (Miller et al., 1988). Microsatellite loci were amplified using multiplex PCR reactions as described in Seddon et al. (2014). PCR fragments were separated by capillary electrophoresis and alleles assigned in GeneMapper (Applied Biosystems, CA).

Identical genotypes were determined using the Microsoft Excel plug-in Microsatellite Toolkit (Park, 2001). Validation that each set of matching genotypes represented a single dugong was performed based on ancillary biological data collected for each animal. The strongest possible biological validation of a genetic match was the presence of a numerically-coded turtle tag on the individual, and when tag numbers matched, matching genotypes were accepted immediately as the same individual. When it was not possible to use inserted turtle tags to determine the validity of a genetic match (i.e., through tag loss or if sampling without capture had occurred), sex, body size/maturity class and distinguishing physical features (e.g., injury, permanent scarring) were used. Photographs of the tail fluke were compared for consistent fluke shape, as well as any identifying scars, particularly those scars indicating that a turtle tag or 'cookie' notch may have been applied previously. When individuals within a cow-calf pair were sampled, these pairings were also used to validate matches, as pairs sampled within the same season, or, in the case of young calves, subsequent seasons, should be consistent. The few dugongs for which genotype matches and biological validation were inconsistent, likely due to human error during sampling, recording, or analysis, were discarded prior to analysis.

2.3. Pedigree reconstruction

Pedigree reconstruction was performed using *PR-genie* (Cope et al., 2014), a program designed specifically for the reconstruction of complex multi-generational pedigrees of long-lived wildlife species via maximum likelihood, based on identification of individuals through microsatellite genotyping, and incorporating biological data, i.e., sex and size class, where available. *PR-genie* uses the algorithm of Almudevar (2003): it proceeds by placing all individuals in the population in an optimal ordering analogous to the relative age of the individuals, i.e., if individual A is a parent of individual B, A should appear before B in the ordering because it is "older", and then for each offspring, it chooses the maximum likelihood parents from those individuals that precede it under the ordering. The ordering ensures that the resulting pedigree is valid, in particular by preventing cases where individuals are assigned as their own descendants. Sex and size data restricts the possible parent pairs and the possible orderings respectively. The maximum likelihood pedigree was constructed for the complete dataset of dugongs from MB, HB, GSS and SB once unambiguous genetic identity was verified with ancillary biological data (see above).

2.4. Data analysis

Parent-offspring pairs and triads featuring individual dugongs that had been sampled in multiple locations were extracted from the reconstructed pedigree, and their characteristics were summarised. The number of links within and between locations were calculated, and the distribution of offspring for parents within each location was determined.

A suite of simulated population systems was developed to provide a baseline from which movement rates could be determined. This was necessary due to the unbalanced sampling proportions between locations in this study. In each simulation, two populations were generated, one containing 883 individuals, and the other 2116 individuals, based on recent population estimates from MB and GSS-HB (combined) (Sobtzick et al., 2012). Individuals within these populations are born, mature, breed, and die based on algorithms designed to mimic the life-history parameters of wild dugongs (after Marsh, 2002). Individuals were allowed to migrate to the other population each season, and the rate at which this occurred was controlled and varied across simulations between 0.01% per season and 10% per season, to provide baseline data for a wide variety of possible movement rates. After a fixed number of seasons, each population was 'sampled' with a frequency equivalent to those observed in this study, i.e., 630 individuals from the smaller population and 340 from the larger population. From the samples within these populations, pedigrees were reconstructed using the same method as was applied to the wild dugong population, and from these pedigrees, summary statistics were calculated: (a) the number and proportions of parent-offspring relationships in the sample that occurred within and between populations, and (b) the number and proportions of parents in the samples for whom offspring existed within the same location only or were also in the other location. These summary statistics were compared with the observed dugong pedigree data to determine likely overall rate of movement between locations. In particular, when the value of a summary statistic for the dugong population was observed to be within the central two quantiles of the distribution of that summary statistic for simulated populations with a given movement rate, that movement rate was determined to be within the feasible range for the dugong population. The comparison of reconstructed pedigrees of simulated populations with reconstructed pedigrees of the wild population allowed inaccuracies in pedigree reconstruction to be taken into account.

Processing of output text files was performed using scripts written in Python 2.6.2 (available at http://www.python.org) and pedigrees were visualised using Graphviz 2.24 (available at http://www.graphviz.org) and Circos (Krzywinski et al., 2009). Statistical analysis was performed using R 2.12.1 (R Core Team, 2012), with a two-sample test of proportions used to compare proportions of relationships involving movement between groups and an exact binomial test to compare sampled groups by sex.

3. Results

3.1. Overall population statistics

A total of 1969 tissue samples from dugongs sampled in Moreton Bay (MB, n = 1369), the Great Sandy Straits (GSS, 488), Hervey Bay (HB, 83) and Shoalwater Bay (SB, 29), were genetically analysed. After genotype matching and biological verification, 1002 individual and unique dugongs were identified unambiguously, i.e., with no recaptures or with consistent genetic and biological data across recaptures (Table 1). This represents approximately 28% of the most recent total dugong population estimate for southern Queensland (Sobtzick et al., 2012). Based on 2011 population estimates (Sobtzick et al., 2012), sampling proportions were 71% of the MB dugong population and 16% of the (combined) HB-GSS population. Of the 1002 individuals, 605 had been sampled once, i.e., represented by a single genotype in the initial dataset, while the remaining 397 were recaptured individuals, with multiple recaptures common and one individual recaptured seven times. The samples from the population included 169 calves, 214 juveniles/subadults and 619 adults. A small number of individuals had either unknown sex, i.e., molecular sex assignment was inconclusive and visual assignment was not possible, or unknown size, where visual estimates were not informative, e.g., when the same individual captured multiple times was assigned different size classes in a way that was biologically unreasonable.

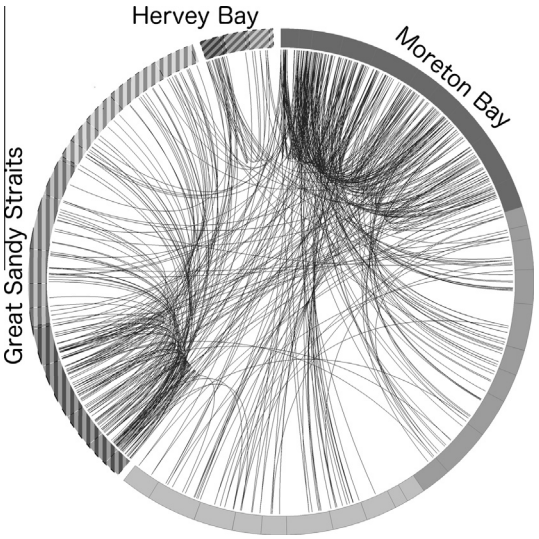

Fig. 1. Parent-offspring relationships between individuals sampled in Moreton Bay (solid), Great Sandy Straits (horizontal stripes), and Hervey Bay (diagonal). Each line represents a parent-offspring link in the pedigree. Within each population, individuals are sorted between adults, subadults and calves (clockwise, dark to light shading, respectively), and chronologically by sampling date within those cohorts, i.e., within each population and size-class group individuals sampled more recently proceed clockwise.

3.2. Pedigree-based movements

A total of 525 parent-offspring relationships were assigned, with 131 individuals assigned both parents and 265 assigned one parent. Most (n = 414, 79%) assigned relationships occurred within the same population, and between individuals sampled as adults (Figs. 1 and 2). Very few individuals that were initially sampled in the study as juveniles or sub-adults were assigned offspring; when this occurred, these were likely individuals that were sampled early in the ten-year sampling program, reached maturity, and then gave birth before their offspring were later sampled.

Six individuals were physically captured migrants, i.e., they were encountered and genetically tagged in multiple locations. Of these, MB02074 was assigned both parents and one offspring; all of these related individuals were sampled in MB, but this individual was sampled three times in MB and then once in HB, five years after his latest capture in MB. GS08225 was sampled three times in GSS then once in HB, and was assigned as the offspring of an individual sampled in MB. The remaining four individuals were not assigned parents nor offspring: of these, one was sampled

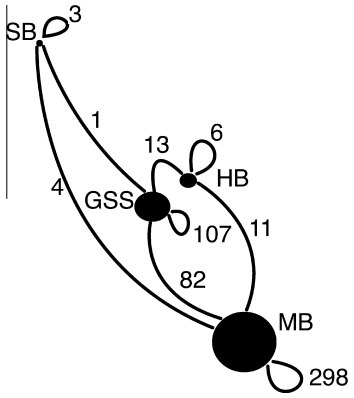

Fig. 2. The number of individual parent-offspring relationships (including as part of parent-offspring triads) assigned within (small loops) and between (connecting lines) locations within southern Queensland: Moreton Bay (MB), Great Sandy Straits (GSS), Hervey Bay (HB) and Shoalwater Bay (SB). The size of nodes represents number of individuals sampled in each location, and the position of nodes give approximate geographical distance between locations.

Table 1
Demographic parameters (sex, size-class) of live individual dugongs sampled in each location: Moreton Bay (MB), Great Sandy Straits (GSS), Hervey Bay (HB), Shoalwater Bay (SB), or recaptured in multiple different locations, over the period of 1998–2011.

Location	Recapture status	Total	Male	Female	Unknown	Adult	Subadult	Calf	Unknown
MB	Single captures	341	136	176	29	203	72	62	4
	Recaptures	289	126	157	6	180	70	38	1
	Total	630	262	333	35	383	142	100	5
GSS	Single captures	179	66	93	20	104	38	36	1
	Recaptures	102	37	60	5	60	23	15	4
	Total	281	103	153	25	164	61	51	5
HB	Single captures	59	29	30	·	33	8	14	4
	Recaptures	10	5	5	·	7	1	1	1
	Total	59	34	35	7	40	9	15	4
SB	Single captures	26	·	5	21	21	·	5	·
	Recaptures	1	·	·	1	1	·	·	·
	Total	27	·	5	22	22	·	5	·
Multiple locations	·	6	4	2	·	3	2	·	1

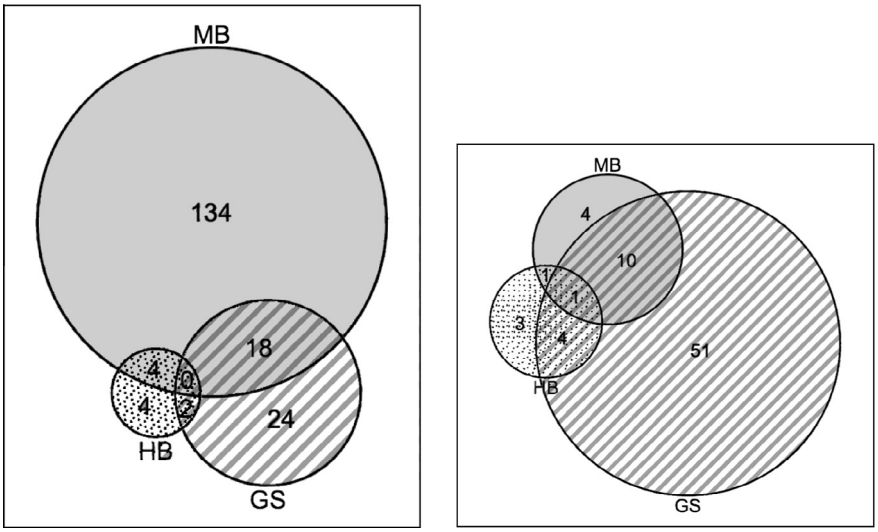

Fig. 3. Source of assigned offspring of parents sampled in (left) Moreton Bay (MB, solid) and (right) Great Sandy Straits (GS, striped), with some offspring existing in Hervey Bay (HB, stippled). Intersecting circles indicate that the parent in question was assigned offspring that were sampled in each location. Shoalwater Bay (SB) individuals not included.

once in each of GSS and SB, one in GSS followed by HB, one in HB and GSS in the same year, and one in MB and later in GSS.

There were 131 individual offspring for whom both parents were assigned, forming a parent-offspring triad. In 40 of these triads, three individuals were not sampled in the same location: in 33 of these triads, one parent was observed in a different location to both the offspring and the other parent. Of these, 11 had the male parent and offspring in the same location, and 22 had female parent and offspring in the same location, i.e., in the majority of cases (significantly more than half, t-test, $p = 0.04$) the male parent was found in a different location. The individuals assigned as offspring were sampled as calves in 14 of the triads, subadults in five and adults in the remaining 14, and the majority ($n = 20$) included the offspring and one parent sampled in GSS, and the remaining parent sampled in MB. None of the 40 triads in which parents and assigned offspring were sampled across locations involved individuals from SB.

In MB and GSS, the majority of parents were assigned offspring that were only sampled in the same location as their parent (Fig. 3), with no evidence to suggest that the proportion of these same location parent-offspring groups was different between populations (2-sample t-test, $p = 0.66$). Sample sizes in HB and SB were too small to be presented in this way.

While most dugong parents had only one or two offspring identified, there were 60 individual adults (33 female, 27 male) for whom three or more offspring were assigned (Fig. 4). Of these,

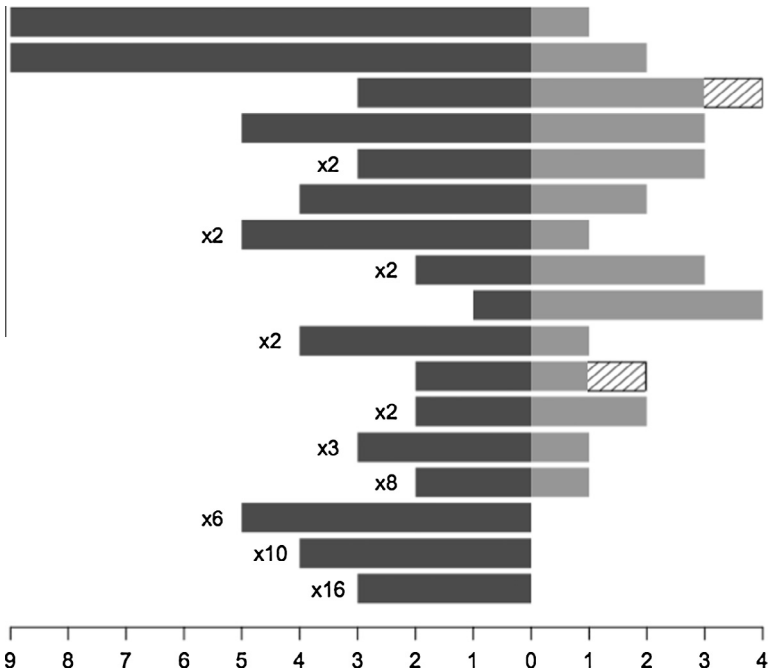

Fig. 4. The distribution (number of offspring) between locations of assigned offspring for those individual parents with ⩾3 assigned offspring. Solid dark bars indicate the number of assigned offspring that were sampled in the same location as the parent, light grey and shaded bars indicate those offspring sampled elsewhere. Quantities beside bars indicate the number of parents with offspring distributed in this way; when not stated there was only one such group.

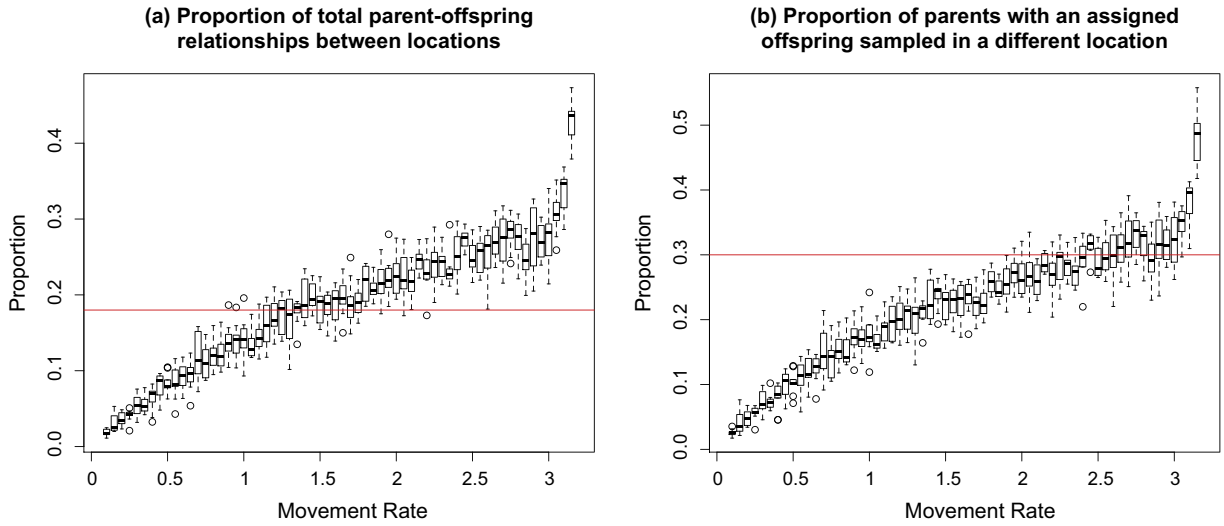

Fig. 5. Comparison between reconstructed dugong pedigree data and reconstructed pedigrees from simulated population systems with a range of annual movement rates, between 0.1% and 3% of the population moving per year. The final three bars indicate migration rates of 4%, 5% and 10%. The left plot shows the proportion of total parent-offspring relationships that occurred between locations within simulated populations. The right plot shows the proportion of individual parents for whom an offspring was sampled in a different location within simulated populations. The red lines indicate the values of these parameters (0.18 and 0.3 respectively) for the reconstructed dugong pedigree. The reconstructed dugong pedigree was considered similar to the simulated populations for a given migration rate when its parameter value (i.e., the red line) fell within the middle two quantiles. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

32 (54%) were assigned offspring found in the same location only, the remainder were also assigned offspring elsewhere. In general, most of the individuals for whom many offspring were assigned had the majority of these offspring sampled in the same location as the parent, but with a smaller proportion sampled in other locations.

3.3. Comparison with simulation

In order to estimate the proportion of the population that is dispersing between locations, observed data were compared with simulated data with different rates of seasonal movement (Fig. 5). The proportion of relationships assigned within and between MB and GSS-HB (combined) in the reconstructed pedigree was similar to that of simulated populations with between 1.15% and 1.75% percent of the population moving per year, as the observed proportion of relationships assigned fell within the first and third quantiles of the simulated data in this range. The proportion of parents in the reconstructed pedigree for whom assigned offspring were sampled in locations other than where the parent was sampled were similar to those of simulated data with annual movement rates between 2.15% and 3% of the population.

4. Discussion

We have demonstrated that the analysis of pedigree relationships in a wildlife population can provide indications of movement and breeding events at an individual level. Pedigree reconstruction is a novel means of detecting contemporary movements in wildlife populations, capable of detecting movements outside the duration of the study, and without the need for physical tracking or the direct observation of individuals in multiple locations. Moreover, pedigree reconstruction provides information about breeding events in addition to movement, i.e., gene flow, information which is not able to be elucidated from telemetry or direct recapture. The utility of this approach is broadly applicable, but of primary use in those cryptic wildlife species for which observation and sampling is challenging and/or expensive, and where individuals are not easily discriminated visually. The dugong populations of southern Queensland, used here as an example of the technique, meet all of these criteria; even after this significant long-term study only a handful of multiple-location recaptures have occurred, but pedigree reconstruction has provided clear indications of movements between locations, far beyond those detectable by multiple-location recapture.

4.1. Summary of dugong data

Of the dugongs in south-east Queensland, only a few individuals ($n = 6$, of a total sampled population of 1002) have been physically recaptured, i.e., genetically tagged in multiple locations during the course of the study, which indicated that some contemporary movement between locations had taken place based on direct observation. However, it was difficult to determine the significance of these movements between populations, due to uneven and incomplete sampling across locations. In contrast, the use of pedigree reconstruction has indicated much higher levels of movement and gene flow between the dugong populations in southern Queensland than has been possible from direct recapture. Substantial numbers of pedigree links between populations along a 600 km coastal strip were detected, with approximately 30% of parents sampled in Moreton Bay (MB) and the Great Sandy Straits (GSS) having at least one offspring that had been sampled in a different location. Whilst this same dataset detected movement of only six individuals by direct recapture, movement in 78 individual parent-offspring groups was detected through pedigree analysis. Approximately 18% of the total number of parent-offspring relationships identified were between individuals found in different locations. The discrepancy between the number of moving individuals for which movement was detected directly, and the number of individuals for which movement was inferred, is due to the difficulty of directly detecting movements. These movements are only detected when the same individual is sampled in each location, and can only detect movements that occurred between when the two samples were taken. In contrast, pedigree methods require both the parent and the offspring to be sampled, but allow for movements that may have occurred at any point over the lifetime of the offspring, providing a much longer window for detection.

Naively, the proportions of individuals that 'stayed home', so to speak, i.e., only having offspring sampled within the same location (and not considering those individuals with no offspring assigned), were the same between MB and GSS. Proportionally more pedigree links were found between GSS and Hervey Bay (HB) (12% of parents from GSS had offspring in HB, 33% of parents from HB had offspring in GSS) than between MB and HB (5% of parents from MB had offspring in HB, 16% of parents from HB had offspring in MB), which is biologically plausible (though not statistically significant) given that GSS is geographically closer to HB, and that dugongs in these locations are often treated as a single population (e.g., Tikel, 1997; Sobtzick et al., 2012). There were too few data to confidently draw conclusions regarding the level of movement between these populations and the more northern SB. We suggest that this link between geographic distance and inferred movement may have been more apparent if more data were available from HB.

4.2. Sampling variability

One concern in this type of analysis is the variable proportions of the populations that have been sampled, i.e., ~70% of individuals in MB against 16% of individuals in HB-GSS (based on population estimates in Sobtzick et al. (2012)), and the possible effect that this may have had on detected relationships and movement events. When comparing relationships between individuals sampled in the same location, this is not a problem. If it can be assumed that the sampling of individuals is independent from their being a parent or an offspring of an individual in the population (i.e., if sampling is unbiased), then a simple Bayesian argument indicates that sampling frequency does not affect the probability of assignment of relationships within a population. This is, however, not as strong an argument in practice as it is in theory, as it is possible that a particular subset of the population may vary randomly from the population parameters, and that variation is more significant with low sampling proportions. In other words, when not much of the population is sampled, you might be lucky and sample proportionally more parent-offspring pairs than in the overall population, or be unlucky and sample fewer. Further, it is possible that the assumption of independence is not actually true, as there may be some inherent sampling bias caused by this sampling strategy, e.g., cow-calf pairs of dugongs being sampled together increases the probability of having both parent and offspring in the sample. We note that in the populations considered here, a similar proportion of sampled individuals from MB and GSS were assigned as parents (30% versus 27%), which reinforces the fact that there is little bias within these populations due to the smaller sample size in GSS. A more complete understanding of the social structure and cohesiveness of dugong populations would allow us to determine these possible biases more thoroughly.

Differences in sampling proportion, however, are confounding when considering between-population comparisons, such as the distribution of offspring for those individuals who were assigned many offspring, or even the proportion of relationships that occurred within rather than between locations (Fig. 2). For an individual sampled in a location with lower sampling proportion (e.g., GSS), a parent-offspring relationship including them was more likely to be observed if the other individual in the pair was present in a location with a higher sampling proportion, i.e., MB. For example, consider a hypothetical individual offspring sampled in GSS: if their parent exists in GSS, they have been observed (and thus can be assigned as parent) with probability $\leqslant 0.3$; but if their parent exists in MB, they have been observed with probability ~0.71. For this reason it was not possible to directly estimate the true proportion of relationships that occur within and between locations with the unbalanced sample proportions considered here, and so simulation was used to provide an indication of overall movement rates. Similarly, the offspring of particular parents were more likely to be observed if they were present in a location with a higher sampling frequency. Each individual assigned more than five offspring had at least some offspring identified in multiple locations. Since sampling proportions were higher in MB than GSS, it is likely that for any particular parent, proportionally more of their offspring in MB were found than those in GSS, e.g., if an individual actually has five offspring in each of these two locations, perhaps four in MB have been sampled but only one in GSS.

The clearest indication of likely movement patterns on an individual level was with individuals for whom both parents could be assigned, and one of the triad was encountered in a different location. These triads both provide more information than parent-offspring pairs and are the most likely of all relationships in a reconstructed pedigree to be true. When one parent and its offspring were in a particular location and the other parent elsewhere, it is more likely that the other parent moved after mating. When both parents were in one location and the offspring was in a different location, it is similarly parsimonious to suggest that the offspring dispersed. Under these assumptions, the 40 instances of these triads are particularly informative, noting that the majority of such cases involve movement by the male parent. This strongly suggests that breeding adult males are more likely to move between locations than breeding adult females, which is consistent with previous indications of possible male-biased gene flow (McDonald, 2006) and roaming of males away from herds during the mating season (Burgess et al., 2012b). These triads may also be influenced by the differences in sampled proportions of the populations: the majority of triads in which an offspring and one parent were found in one location and the other parent in a different location included the offspring with one parent in GSS and the other parent in MB, presumably due to the differences in proportions of each population that were sampled. If a parent and offspring were sampled in one location and the other parent existed in a different location, they were more likely to have been sampled if that other location was MB. It is also important to note that in many of these triads, the offspring were sampled as young calves with their mothers. Since cow-calf pairs remain together for several years after birth, it is likely that if they move during this period, they would do so together. However, there are few data on the movement of these cow-calf pairs, as they have not been directly recaptured in multiple locations (this study) and only limited telemetric tracking has occurred (Sheppard et al., 2006), so that movement patterns are unknown, and thus it is not certain that the location in which these calves were sampled was the location that they were born.

4.3. Overall movement rates

Comparison of the reconstructed dugong pedigree with pedigrees of simulated populations under varying levels of movement indicated that the proportion of pedigree relationships between populations observed in dugongs were similar to those scenarios in which 1–3% of the population moved each year. It is important to note that within these simulated scenarios not all dispersing individuals were involved in breeding events. Variation in inferred movement rate between the two methods was likely due to imperfect simulation modelling, e.g., simulating movements as random when in the wild there may be some unknown driving factor, or due to random variation based on incomplete sampling. This 1–3% movement rate is higher than expected based on previous analyses of population structure (Seddon et al., 2014). There is evidence of long-range movements of dugongs between foraging grounds through direct recapture (this study) and telemetry (Sheppard et al., 2006). Telemetric data have demonstrated movements of

more than 100 km by 14 of 70 tracked dugongs and ranges of up to 560 km (Sheppard et al., 2006). There are also a few recorded incidences of dugongs found outside their normal foraging locations and in areas without extensive seagrass meadows, such as on the exposed Sunshine Coast, located between the MB and HB regions (Marsh et al., 2001a). These dugongs were likely in transit, providing further evidence of movements between locations.

It is not clear if the high level of inferred movement here, approximately 1–3% of dugongs moving each year, equates to gene-flow between populations within southern Queensland, which may be important for the maintenance of genetic diversity especially if populations drop to low levels. However, this level of dispersal may be inconsistent with the low but significant population differentiation noted on microsatellite analysis (Seddon et al., 2014), suggesting that not all movement inferred here is effective genetic dispersal, i.e., not all individuals that move are breeding after moving to a new location. The observed genetic relationships between sampled individuals could feasibly be the result of regular, low level movement, but large scale movements cannot be ruled out on pedigree data alone. Regular low-level movements, particularly combined with indications that males are more likely to move and breed, could provide indications of some dispersal of males at maturity or movement as a response to mating competition. Movement events where a large proportion of a population moved at once, if they occurred, may occur in response to environmental disturbances, either natural or anthropogenic (Marsh and O'Shea, 2012). The most significant recent major ecological disturbance in this region was a flood event in early 2011, resulting in widespread damage to seagrass beds and thus shortage of forage for dugongs. Subsequent to this, aerial surveys indicated declines in population levels in HB-GSS and increases in MB (Sobtzick et al., 2012) (compared to aerial survey results from 2005), suggesting a possible large-scale migration event. However, two lines of evidence suggest that such a large scale movement event probably did not occur. Firstly, population estimates based on capture-mark-recapture rather than aerial survey indicated a population size of 940 ± 75 in 2009 (Lanyon et al., 2009b), and based on this higher estimate, population levels in MB did not appear to increase in 2011. Secondly, intensive sampling of 177 individuals (20% of 2011 population based on aerial survey estimates due to Sobtzick et al. (2012)) in MB that occurred in the 10–11 months after the 2011 flood event did not detect individuals that had been tagged previously in other locations. Given a population estimate of 883 in MB and 340 tagged individuals in Hervey Bay (HB) and GSS, the probability of seeing no HB-GSS individuals from 177 samples if 10% of the HB-GSS population had moved to MB due to the disturbance is 9×10^{-4}, i.e., it is unlikely that no dugongs previously sampled in HB-GSS would be sampled in MB if there had been a substantial migration event. This suggests that population fluctuations observed in HB and GSS were likely not the result of large-scale movement to MB, but rather may have been due to movement elsewhere, i.e., into deeper water offshore (Sobtzick et al., 2012), some mortality, and/or artifactual error associated with aerial survey techniques (Lanyon et al., 2003; Seddon et al., 2014).

4.4. Trends and analysis of individual behaviour

Pedigree reconstruction techniques detect a significant proportion of relationships that exist in a population (Cope et al., 2014) and as such provide insight into general contemporary movement trends between populations. However, appropriate care must be taken not to read too much into the implied behaviour of any individual. Pedigree reconstruction is not without challenges: given finite genetic data, and without reliable relative age data for all individuals, a maximum likelihood pedigree will not necessarily be the true pedigree. Individuals may be assigned to parents or offspring incorrectly, or true relationships between individuals may not be detected. When the true parent of an individual exists in the sample, they will likely be assigned correctly approximately 70% of the time, given 25 microsatellite loci with average expected heterozygosity ~0.5 (Cope et al., 2014), as is the case in this study. In practice, for this study, this means that some inferred relationships will be incorrect, so available biological data should be considered carefully when interpreting any particular relationship. However, the simulated populations from which comparisons were drawn shared similar genetic characteristics and thus overall estimates of movement based on these simulations take the inaccuracy of pedigree reconstruction into account. It would certainly be desirable to have substantially higher accuracy than this, but it provides a baseline from which trends can be drawn. To improve the accuracy of pedigree reconstruction, a larger number of markers of greater variability (i.e., higher heterozygosity) would be needed. In some sense the insight provided by pedigree reconstruction is the opposite of what is learnt from physical recapture in multiple locations, as physical recaptures come with high certainty regarding the movements of particular individuals, but unless these recaptures are very frequent they provide little insight into overall movement trends.

While it is safer to consider general population trends given the potential uncertainty in reconstructed pedigrees, it is still possible to attempt to construct narrative timelines for the movements of individual dugongs based on their recapture histories and those of assigned parents or offspring. When the assigned offspring in a triad were calves, this gave some indication of when individual dugongs may have moved, e.g., one triad included a mother and calf sampled in HB in 2010 and a father sampled in MB in 2001, indicating that the male may have moved to HB in the interim. The alternative scenario, of a movement of cow and calf or pregnant cow may be less likely. Another triad included a mother and calf sampled in GSS in 2006 and a father sampled in MB in 2001, (March) 2003, and (September) 2005, indicating that the male may have moved to GSS and returned to MB in 2002 or later in 2003. When multiple offspring and recaptures are involved, the complexity of movement analysis concerned individual MB98007, who was sampled in MB in each of 1998, 2005 and 2008 and assigned as father to three individuals in GSS and two in MB. The three offspring in GSS were all observed as sub-adults in 2009, with different mothers. It is unclear if this father was present in GSS prior to 1998, or between 1998 and 2005; This could only be clarified if the absolute or relative ages of the subadult offspring could be determined, as dugongs in southern Queensland may not reach adulthood until their teenage years (Burgess et al., 2012a,b) and thus the dugongs sampled as subadults in GSS in 2009 may have been sired as early as the 1990s. In general, care needs to be taken as it is not possible to be certain where an individual was born, nor of the extent or timing any possible movements between recaptures, i.e., if an individual was captured, then moved elsewhere but returned before a subsequent capture.

When individuals identified as being involved in movement events via pedigree methods are analysed with alternate methods, results are often consistent. These same dugong populations in southern Queensland were analysed for genetic differentiation using the Bayesian clustering method STRUCTURE by Seddon et al. (2014), and two population groups were subsequently identified, corresponding predominately to one MB based group and one GSS-HB-SB group. Those individuals found here to have offspring in multiple locations were often assigned to different STRUCTURE population groups than would be expected given their observed location, or were assigned mid-range probabilities of group membership. For example, MB98007 mentioned above as having been sampled multiple times in MB but with multiple offspring in GSS, had probability 0.945 of being a member of the

GSS-HB-SB group. MB08706, assigned a parent and offspring in GSS but sampled in MB with a calf, had 0.55 of being in the MB group, i.e., was only slightly more likely to be in this group than GSS-HB-SB. Other individuals with offspring in multiple locations such as MB02065 (five MB offspring, four GSS), MB02097 (two MB offspring, two GSS) and MB07600 (three MB offspring, three GSS) each had high (>0.8) probability of being members of the GSS-HB-SB group, whilst MB01001 (four MB offspring, two GSS) had 0.55 probability of belonging to the MB group. The combination of these two techniques may give greater insight into which individuals may have moved, but care must be taken as the individuals themselves (through their offspring) contribute to the genetic makeup of the population groups in question.

4.5. Conclusion

We have demonstrated that pedigree techniques provide a means of detecting movement and breeding events at an individual level in populations where direct observation of these movements is difficult and detection of movement from capture-mark-recapture is rare. For the dugongs in southern Queensland, Australia, markedly more movement between locations was detected through pedigree reconstruction than has been previously possible through direct recapture of individuals in multiple locations or through telemetry studies. Population-level indications of movement are clear, and provide particular insight when considering familial triads for which both parents are assigned to an offspring. Unfortunately with the data available here it was not possible to determine, on a population level, the impact of past environmental stressors on frequency, extent and timing of movement between locations.

Pedigree links may, with care, provide insight into the movements and breeding events of individuals, and when combined with time-series recapture data may give possible insights into historical movements over the lifetime of individuals. These techniques can either supplement gene-flow based methods or provide insight into dispersal even when gene-flow based methods are difficult. Pedigree-based techniques for the analysis of movement and breeding events that occur amongst wildlife populations have broad applicability and will allow for deeper understanding of the behaviour of these species for which direct observation of movement and breeding is difficult.

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