Population structure of short-finned pilot whales in the oceanic archipelago of Madeira based on photo-identification and genetic analyses: implications for conservation

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ABSTRACT

1. Pilot whales Globicephala spp. are known to display a hierarchical social pattern, but longitudinal data to infer population structure of short-finned pilot whales Globicephala macrorhynchus are rare.

2. Using data collected between 2003-2011 in the oceanic archipelago of Madeira, the grouping structure of short-finned pilot whales was studied using photo-identification methods and mtDNA sequences and microsatellite markers to test the hypotheses that (1) there is at least one pelagic and one or more island-associated communities, and (2) groups are made of related individuals, with a matrilineal social structure.

3. Pilot whales demonstrated a large degree of variability in site fidelity, including residents (up to 14-year interval), regular visitors and transients. The social and temporal analyses revealed a well-differentiated society with long-lasting relationships (of years). The genetic analyses suggested that individuals of the three residency patterns may not be genetically isolated, and that small groups are made up of related individuals, suggesting some degree of social philopatry, while large groups are probably temporary associations of smaller groups.

4. It is proposed that the pilot whales encountered in Madeira belong to a single population encompassing several clans, possibly three clans of island-associated whales and others of transients, each containing two to three matrilineal pods, each with a mean of 15 individuals (SD=9, range: 4-29). We suggest that the clans interact for mating purposes when they meet.

5. For management decisions, it is considered that the island-associated whales should not be regarded as demographically independent populations, but instead as stable social entities to be included in governmental management plans and requiring periodic evaluation of their status. The high proportion of marked individuals and low rate of mark change encourages further research in this species.

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entities and influence the definition of management policies. Photo-identification (photo-id), which is based on the repeated identification of individuals, has become an essential technique in providing information on group stability and association patterns in longitudinal studies (Würsig and Würsig, 1977; Würsig and Jefferson, 1990). Examples include the intensively studied resident killer whales (Orcinus orca) where natal pod philopatry has been evidenced in both sexes (Bigg et al., 1991a, b, 1993a, b; Connor, 2000), and the long-finned pilot whales (Globicephala melas) with non-random social systems based on constant companions (Ottensmeyer and Whitehead, 2003; de Stephanis et al., 2008).

In the case of the short-finned pilot whales (Globicephala macrorhynchus), photo-id was described as a feasible technique for studying their social organization (Miyashita et al., 1990; Shane and McSweeney, 1990), and Heimlich-Boran (1993) found support to the hypothesis that this species has a matrilineal hierarchical system. Even though, up to date, and despite this species being distributed worldwide from tropical to warm-temperate waters and considered abundant throughout its range (Olson, 2009), behavioural longitudinal studies on this species using photo-id are scarce; only Heimlich-Boran (1993) and Mahaffy (2012) have analysed large datasets (of hundreds of individuals) over multiple years.

Another technique that has become widely used for the analysis of population structure in free-ranging cetacean species is molecular genetics. It has been revealed to be of great importance in identifying conservation units and estimating gene flow between demes (cf. Hoelzel et al., 2002). Molecular analyses have also been used to obtain information on social and geographic philopatry in several delphinid species, such as bottlenose dolphins (Tursiops aduncus, Wiszniewski et al., 2010), killer whales (Pilot et al., 2010) or striped dolphins (Stenella coeruleoalba, Gaspari et al., 2007). In most toothed whale species, females form stable matrilineal lineages as a result of social philopatry, while males either disperse (e.g. sperm whales, Physeter macrocephalus) or display geographic philopatry (e.g. bottlenose dolphins) or even social philopatry (e.g. long-finned pilot whales and resident populations of killer whales, Amos et al., 1991a, b, 1993a, b; Connor, 2000).

To our best knowledge, inference on the genetic differentiation of short-finned pilot whales is confined to the studies of Wada (1988) and Oremus et al. (2009). While the former found two genetically isolated forms off the Pacific coast of Japan, the latter analysed worldwide population structure, finding strong mtDNA differentiation between oceanic basins and low haplotype and nucleotide diversity compared to other abundant widespread cetaceans. Nevertheless, and contrarily to its related species (see Amos et al., 1991a, 1993a; Fullard et al., 2000), very little information is available on the population structure of short-finned pilot whales either for the Atlantic or for offshore archipelagos.

The pelagic waters of the archipelago of Madeira are amongst the most isolated oceanic habitats of the north Atlantic. Field surveys conducted over the last decade within the frame of several research projects (see acknowledgments) showed that pilot whales are among the four most encountered odontocete species (our unpublished data). However, scientific knowledge on these whales’ ecology and population structure in Madeiran waters is lacking. One study using photographically-identified individuals suggested seasonal site fidelity for Bryde’s whales (Balaenoptera brydei) (Alves et al., 2010). Knowing if short-finned pilot whales in Madeiran waters belong to a single large oceanic population and thus be regarded as a single conservation unit, or if they are divided into multiple populations, could greatly improve future management decisions. One could expect an absence of population structure and genetic isolation given the wide spread oceanic habitat surrounding the archipelago and the pelagic nature of this species, which is known to undertake large-scale movements (Bernard and Reilly, 1999). Nevertheless, the presence of island-associated whales in Madeira should be regarded as a possibility, based on a preliminary photo-id analysis (Alves et al., 2007) and on the occurrence of resident pods of this species (Heimlich-Boran, 1993; Mahaffy, 2012).

The present study is the first one to use a combination of photo-id and molecular genetics to determine whether individuals associate with kin and to infer the population structure of pilot whales. In the archipelago of Madeira, the grouping structure of short-finned pilot whales was studied using individually recognizable whales, in order to assess site fidelity, residency and association patterns between 2003-2011. Additionally, mtDNA sequences and microsatellite markers were used to estimate inter-individual relatedness within and between groups of whales encountered during 2004 and 2005. We hypothesized that (1) there is at least
one pelagic and one or more island-associated communities, and (2) groups are made of related individuals, with a matrilineal social structure. Finally, we expected to contribute to the establishment of effective conservation measures as part of a long-term governmental management plan.

METHODS

Study site

The study was conducted around the warm-temperate islands of Madeira, Desertas and Porto Santo, archipelago of Madeira (Portugal). These oceanic volcanic islands lie at 670 km from Morocco, and steep submarine canyons and deep waters (ca. 1500 m) are common around them due to a reduced continental shelf (Geldmacher et al., 2000). The study area covered a total of 4818 km² up to 20 km offshore around the three islands. However, since data were obtained through different sources, a more intensely surveyed core area of ≈900 km² was defined along the south and east of Madeira Island, up to 15 km offshore (Figure 1).

Field procedures

Individual identification photographs were obtained through year-round systematic and non-systematic surveys, as well as from other sources. One hundred and six systematic surveys (with a mean duration of 11 h 30 min) were carried out in the whole study area following predefined line-transects using the 18 m research-yacht *Ziphius* (12 km h⁻¹) during 2004 and 2007-2011. Here, time constraints have limited full photographic coverage of some encounters. One hundred and seventy non-systematic surveys (with a mean duration of 6 h 16 min) were carried out mainly in the core area using the 6.5 m Rigid Inflatable Boat *Roaz* (15-25 km h⁻¹) between 2004-2011. All groups of short-finned pilot whales seen were approached and attempts to photograph the left- and right-side of all individuals were made, independently of age class and of how distinctive a dorsal fin was.

A mean of three observers searched the area in Beaufort sea state ≤3, and track courses were recorded using a GPS. Weather, effort and sighting information (location, initial time, best estimate of group size and composition) were recorded. A group was defined as all individuals within a 250 m radius of each other and exhibiting similar behaviour (Heimlich-Boran, 1993). An encounter was defined as a sampling event that provided at least one photographic capture.

Photo-id

Members of the research team took photographs using digital cameras with lenses ranging between...
70-400 mm. Additionally, digital photographs from other photographers taken between 2003-2011 onboard whale-watching boats that operated year-round in the core area were also analysed, and film photographs taken by our team during opportunistic surveys in 1997 and 2001 at south Madeira underwent individual matching examination to help determining long-term site fidelity. A photo-id catalogue was compiled and a dataset of capture histories per encounter was created using the individual identification photographs (Würsig and Jefferson, 1990). A capture was defined as an individual identification within an encounter.

Photo processing consisted of cropping the dorsal fin and the surrounding body area, and adjusting contrast with Adobe Photoshop Elements 3.0. The result was a processed image that highlighted the trailing edge, thus facilitating sorting and matching (Figure 2). Photo matching consisted of comparing processed images based primarily on the number of unique notches on the dorsal fin, which allowed matching left- and right-side photographs. A notch was considered as an indentation in the leading or trailing edge of the dorsal fin, identifiable from both sides (Auger-Méthé and Whitehead, 2007). Other secondary features such as fin shape or scars (Auger-Méthé and Whitehead, 2007) were only used to sort individuals without unique notches, as well as to confirm matches. At the end, all matches were double-checked and all individuals compared in order to avoid false-positives and false-negatives. The photo-id was done by the same person (FA), and the long-term matches were confirmed by a second researcher experienced in photo-id (AD).

A photo quality to the best image of each capture and a distinctiveness category to each catalogued whale were assigned following an independent 1-4 rating (Baird et al., 2009); consisting of poor, fair, good or excellent quality, and non-distinctive, slightly distinctive, distinctive or very distinctive, respectively (Figure 2). The photo quality rating was based on focus, clarity and contrast of the image, and on the angle and size of the dorsal fin relative to the frame (Friday et al., 2000). Captures featuring photo quality 3-4 were defined as high-quality (HQ) images. Non-distinctive individuals were based only on scars or on atypical fin shapes, slightly distinctive individuals had few small notches along the trailing edge, distinctive individuals had multiple notches or at least a big one in the dorsal fin, and very distinctive individuals had similar types of markings but to a greater extent. Individuals with distinctiveness 3-4 were defined as well-marked (WM). Unless otherwise stated, this study used only WM-individuals and HQ-images, thus increasing the certainty of matches. The coverage of the proportion of captures per encounter, calculated as the number of individuals captured divided by the number of individuals in the group, was graded as: low-coverage if this proportion was <0.3, medium-coverage if ≥0.3 and <0.8, high-coverage if ≥0.8 and <1, and full-coverage if =1.

Whales were classified in age categories based on the comparison of the relative size of individuals using photographs featuring multiple individuals as well as using field notes, according to descriptions by Yonekura et al. (1980) and Kasuya and Marsh (1984). This study considered immature whales as (calves or juveniles) individuals that had a lighter (grey) colour without a pronounced melon, and that were <⅔ the length of the adult individual that it was in close association with. Sub-adults were individuals slightly smaller than adults. Due to the difficulty to distinguish between sub-adult males and adult females, the latter were considered only when observed constantly in the presence of an immature.
Photographic analysis

Three residency patterns were considered based on the individual capture histories. Individuals that exhibited multi-year and year-round site fidelity (captured ≥5 times in at least 3 years and 3 seasons, i.e., spring, summer, autumn and winter) were termed residents; individuals captured only once were termed transients; and individuals that fell between these thresholds were considered temporary emigrants/immigrants or regular visitors and thus termed visitors. Exceptions were made in a few cases when individuals were captured only twice within ≤8 days interval, even if in two seasons. These animals were also considered transients since they (1) were probably only passing through, and (2) presented a clear distinct residency pattern from the visitors, that were commonly captured (more than twice) yearly and/or within one or two seasons. Immatures captured only once were assigned the same residency pattern as the respective adults that accompanied them.

The proportion of WM-individuals within groups was estimated using only data from encounters with full-coverage obtained during surveys. A one-way ANOVA and a post-hoc Tukey test were used to check if significant differences existed in the proportion of WM-individuals between groups composed exclusively of residents, of visitors or of transients. Similarly, the proportion of at least slightly distinctive individuals was also estimated to allow comparison with other studies. Estimations included encounters with small (3) and large (33) numbers of individuals, thus not biasing results to sampling only small or large groups, and reducing variance due to not sampling exclusively small groups. To estimate the rate of mark change due to the acquisition of new notches in the dorsal fin over time, the time between the first and last capture for all recaptured individuals was summed and divided by the minimum and maximum number of mark changes that occurred (Aschettino et al., 2011).

Group size was determined at sea using only encounters from surveys, ensuring less biased counts. While the analysis of group size per month used data from encounters of all coverage, the analysis of group size per residency pattern was restricted to encounters with high and full-coverage. Additionally, a group featuring individuals of different residency patterns was termed a mixed group, and in these cases, encounters of all coverage were also used (since a group with a minimum of two individuals of different residency patterns was already considered a mixed group). A two-way ANOVA test was used to check if significant differences in group size existed between months and type of groups (according to residency pattern). To test for a preferential period of occurrence of mixed groups, we used a binomial two-sample proportions test to assess if the proportion of mixed groups (compared to other types of groups, i.e., groups dominated by residents, by visitors or by transients) was significantly different between two predefined periods, i.e., January-June vs. July-December. We used the Shapiro-Wilk and the Levene’s tests for the ANOVA assumptions (α=0.05). All the preceding analyses were carried out using the R 2.14.2 statistical package (R Development Core Team, 2012).

Associations

A social network diagram was created using NetDraw 2.119 (Borgatti, 2002) to illustrate individual association patterns. All encounters were considered, but the ones resulting in a single individual were excluded due to not providing any linking information. Residency pattern was included as an individual attribute.

The simple ratio (Ginsberg and Young, 1992) and the half-weight (Cairns and Schwäger, 1987) association indices were used to represent the strength of the behavioural relationships between dyads, where 0 means two individuals were never recorded associated, and 1 that they were always associated. Considering that the inferences drawn were similar and that the half-weight index is less biased when not all associates are identified (Whitehead, 2008), as it is in the case of this dataset, the results presented here concern the half-weight index.

Hierarchical cluster analysis was used to classify and illustrate relationships between the whales that were captured more often. Only whales captured on ≥4 encounters with high- or full-coverage were used to ensure a more accurate representation. Sampling period as well as associations was defined as individuals grouped within an encounter. Associations were calculated using the average-linkage method due to presenting the highest cophenetic correlation coefficient (CCC) when compared to single-, complete- and Ward’s-linkage (not shown), and also due to being less affected by sampling error (Milligan and Cooper, 1987). Knowing that dendrograms impose a hierarchical structured society for a valid
interpretation (Whitehead, 2008), we considered the CCC (where a value between 0.8 and 1 is a good fit; Bridge, 1993) and tested for preferred/avoided associations using permutation tests (Bejder et al., 1998; Whitehead, 1999). The null hypothesis was that individuals associate with the same probability with all other individuals, given their availability. Observed SD and CV of the pairwise association indices that were significantly larger than those from permuted datasets were taken as evidence that individuals preferred or avoided companions (Whitehead, 1999). To ensure that P-values were stable, 1000, 10000 and finally 20000 permutations were generated, and three runs performed using the simple ratio plus three using the half-weight association index. Modularity-G performed using the simple ratio plus three using the permutations were generated, and three runs were performed using the half-weight association index. Modularity-G (Newman, 2004) and knot-diagram (Wittemyer et al., 2005; Whitehead, 2008) methods were used for population division. The resulting social entities were defined as ‘pods’ (Bigg et al., 1990; Heimlich-Boran, 1993), and an alphanumeric ID was assigned.

Standardized lagged association rates (SLAR) were used to address temporal patterning in social relationships (Whitehead, 1995). To interpret SLAR we also considered the null rates, which were the values animals would have if associated randomly (see Whitehead (1995) for equations). Moving average was chosen as that which best adjust between precision and smoothing. SLAR were plotted on a log x-axis time lag scale since time differences between sampling periods ranged over an order of magnitude. We used data from encounters with high- and full-coverage, independently of the number of captures. Sampling period was defined as day, and associations as individuals grouped within an encounter. The precision (SE) was estimated using the temporal jackknife method on each sampling period (Whitehead, 2008). Exponential models representing simulated social structures (Whitehead, 1995) were fitted to the SLAR (Table 1). According to Whitehead (2008), the best fit model was chosen as that which minimized the Quasi Akaike Information Criterion (QAIC). All association analyses were performed with the compiled version of SOCPROG 2.4 (Whitehead, 2009).

**DNA analyses**

Thirty-two skin samples were collected in September-November 2004 and in February, September and October 2005 using a biopsy darting system (cf. Mathews et al., 1988). Samples were collected from 14 groups, most were from adult individuals, and the number of samples per group did not exceed 33.3% of the group size. Details on sampled groups are given in Table 1ESM (electronic supplementary material).

Samples were stored in 90% ethanol and processed at INETI, Lisbon, Portugal. DNA extractions were performed using the DNeasy tissue isolation kit (Qiagen) following the manufacturer’s protocol. About 1-2 mm³ of skin were minced and rinsed in dd-water. Digestion by recombinant proteinase K was extended overnight at 56°C.

Part of the tRNA-Thr, the tRNA-Pro and the most variable part of the mitochondrial control region were amplified using the primers Dloop-16L (Hoelzel et al., 1991; n = 24 short sequences) and H00034 (Rosel et al., 1994) or Dloop-19H (Hoelzel et al., 1991; n = 5 long sequences). PCR reactions were carried out in a 25 µl volume using 0.75 units of Taq DNA polymerase (MBI Fermentas) in KCl buffer with 2 mM MgCl₂ and 0.3 µM of each primer. The number of cycles was set to 35 and the annealing temperature to 52°C. PCR products were purified with the GFX PCR DNA purification kit (Amersham Biosciences). Sequencing was done on an ABI-prism capillary sequencer (Applied Biosystems) by Macrogen, Korea. All samples were sequenced with the 16L primer, using an annealing temperature of 55°C. Five randomly selected samples were also sequenced with the reverse primer, and amplification and sequencing were

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**Table 1.** Fit of social system models to the standardized lagged association rate (SLAR) for all individuals. τ is time lag in days. CA – casual acquaintances, CC – constant companions. Models ranked by the Quasi Akaike Information Criterion (QAIC); the lowest indicates the best-fitting model. CA is just one interpretation of the SLAR pattern (according to Whitehead, 1995) and not appropriate in this case where we have long-term relationships falling off only after about three years.

<table>
<thead>
<tr>
<th>Description of model</th>
<th>Model formula</th>
<th>Number of parameters</th>
<th>QAIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA</td>
<td>0.092309 e^{(-0.0003214 \cdot τ)}</td>
<td>2</td>
<td>3973.4</td>
</tr>
<tr>
<td>Two levels of CA</td>
<td>4.2545 e^{(-0.00546 \cdot τ) + 0.091674 e^{(-0.0003165 \cdot τ)}}</td>
<td>4</td>
<td>3975.7</td>
</tr>
<tr>
<td>CC + CA</td>
<td>0.069697 + 0.19069 e^{(-0.24605 \cdot τ)}</td>
<td>3</td>
<td>4001.9</td>
</tr>
<tr>
<td>CC</td>
<td>0.071094</td>
<td>1</td>
<td>4006.6</td>
</tr>
</tbody>
</table>
repeated for two randomly chosen samples, and no mismatches were found. All sequences were carefully checked for errors by visual inspection of peak heights with Chromas Lite 2.01 (freeware available on-line at http://www.technelysium.com.au/chromas.html). Dubious nucleotides were systematically verified. Alignment was performed visually and no gap was found. Short sequences were of 609 base pairs (bp) and long sequences of 999 bp.

Eight polymorphic dinucleotide microsatellite loci were analysed. PCR reactions were performed in multiplex, using a touch-down protocol with a temperature decrease of 0.1 °C per cycle over 25 cycles. The initial annealing temperature was set at 52 °C for Sw10 and Sw19 (Richard et al., 1996), and 56 °C for EV37 (Valsecchi and Amos, 1996), FCB1 and FCB17 (Buchanan et al., 1996), as well as for Mk6 (Krützen et al., 2001) and d22 (Shinohara et al., 1997). Locus 468/9 (Amos et al., 1993a) was amplified separately, with a fixed annealing temperature of 45 °C. Fragments were scanned on an ABI 310 capillary sequencer using the size marker ROX350 (Applied Biosystems). Molecular sexing was performed by co-amplification of a short fragment of the male-specific SRY gene (CSY, 157 bp; Abe et al., 2001) and a tetranucleotide microsatellite used as a PCR control for positive identification of females (GATA028; Palsbøll et al., 1997), using a touch-down protocol with an initial annealing temperature of 52 °C. Unsuccessful PCR reactions were repeated up to three times. DNA extraction and genotyping were repeated whenever a sample was found not to amplify at three or more loci. Samples that failed this second attempt (n = 2) were removed from the data set. Three randomly chosen samples were genotyped twice to verify that results were reproducible.

Identification of replicated samples
Genotypes were checked for potential errors and replicated individuals using Microsatellite Tools (MsTools; Park, 2001). Allowing for a maximum of two mismatches between samples, one pair of potential replicates with identical genotypes was identified. Extraction and genotyping was repeated for these two samples. Genotype identity was confirmed and one of the two samples was removed from the dataset prior to analyses. There were also two pairs of individuals differing by three mismatches. The mismatching loci were re-analysed and the initial genotypes were confirmed.

Genetic statistical analyses
As the mtDNA analyses yielded a single haplotype, no statistical analyses were performed on the mtDNA sequences. As for microsatellites, we verified that loci were not in linkage disequilibrium and tested each locus for departure from Hardy-Weinberg frequencies using the exact test implemented in Genepop on the internet (http://genepop.curtin.edu.au/index.html; Genepop 3.3 by Raymond and Rousset, 1995), with default settings. We used a sequential Bonferroni correction to compensate for multiple tests (cf. Rice, 1989). Probability of genotype identity between unrelated and first-order related individuals, allelic diversity, observed (H0) and expected (H E) heterozygosity were calculated with GenAlEx v.6 (Peakall and Smouse, 2006). Despite the small number of samples, an attempt was made to estimate genetic differentiation between resident and transient individuals by means of F ST and R ST calculated with Arlequin 3.1 (Excoffier et al., 2005). Significance was tested by 10000 permutations. Pairwise relatedness coefficients between individuals were calculated using SPAGeDi 1.2d (Hardy and Vekemans, 2002). The coefficient r Q&G (Queller and Goodnight, 1989) was selected for being widely used and readily interpretable. For each residency pattern, we calculated the mean degree of relatedness and its variance following Ritland (2000); significant deviation from zero was tested by 10000 permutations, using SPAGeDi 1.2d (Hardy and Vekemans, 2002).

We plotted the distribution of pairwise relatedness coefficients within and between groups and searched for potential first order relatives (r Q&G > 0.5). We tested whether relatedness was higher within than between groups using a permutation procedure, which allowed accounting for the fact that pairwise relatedness coefficients were not independent from one another using Genetix 4.03 (Belkhir et al., 2001) to perform a Mantel test as suggested by Sokal and Rohlf (1995). We searched for a correlation between a matrix of pairwise genetic relatedness (r Q&G) and a matrix of group membership encoded as 1 for pairs of individuals sampled in the same group and 0 for pairs of individuals not sampled in the same group. The number of permutations was set to 10000. We also tested whether there
was a correlation between mean intra-group relatedness and group size by means of a Spearman’s rank test performed with Statistica 6.1. Finally, using all biopsied individuals that had been photo-identified, we searched for a correlation between pairwise relatedness and their half-weight index by means of a Mantel test with 10000 permutations performed with Genetix 4.03 (Belkhir et al., 2001). Half-weight indexes were calculated for all biopsied individuals that had been photo-identified, independently of the number of times they had been encountered. Only HQ-images and groups with high- or full-coverage were included.

**RESULTS**

**Photo-id**

Over 45000 photographs taken from 405 encounters resulted in 1114 captures and 364 WM-individuals based on HQ-images (Table 2). The mean duration of each encounter, calculated as the time between the first and last photograph, was 27 min (SD = 23, n = 105) during surveys, and 14 min (SD = 12, n = 300) during whale-watching trips; totalling 7014 min of photo-id. The capture frequency histogram shows different capture probabilities; 71.7% of the individuals were captured only once and a subset of 10 individuals presented 25 to 33 captures (Figure 3). The discovery curve slightly stabilized throughout the years but not completely by the end of the study, indicating that not all individuals using the area have been captured (Figure 4). Encounters with 100% of recapture started being common after 2006 (∼350 captures). The sampling effort (number of captures) was higher in 2010 and 2011 (Figure 4).

Of the 364 individuals, 28.3% (103 individuals) were recaptured. Of these, 82.5% (85) were captured in different years. The median inter-annual interval between the first and the last capture for these 85 individuals was 4 years (mean = 3.9, SD = 2.2, range: 1-8) for the study period (2003-2011). For the 103 recaptured whales, individuals captured in 2 years were the most frequent, >10 individuals were captured in 3 and 4 years, <10 individuals were captured in 5, 6 and 7 years, and one individual was captured in 9 years; indicating site fidelity for a subset of individuals (Figure 5). The matching examination of 19 individuals from opportunistic surveys revealed that (1) one individual captured in October 1997 was an individual that had been captured yearly between 2004-2011, thus presenting a 14-year recapture interval, and (2) one individual captured in January and another one captured in May 2001 were recaptured in January 2011 and June 2010, presenting a 10- and a 9-year recapture interval, respectively.

### Table 2. Total number of captures and individuals (in bold) and percentages of their respective categories for high-quality images and well-marked individuals

<table>
<thead>
<tr>
<th>Captures (total number)</th>
<th>1114</th>
</tr>
</thead>
<tbody>
<tr>
<td>Photo quality</td>
<td></td>
</tr>
<tr>
<td>good</td>
<td>44.5%</td>
</tr>
<tr>
<td>excellent</td>
<td>55.5%</td>
</tr>
<tr>
<td>Individuals (total number)</td>
<td>364</td>
</tr>
<tr>
<td>Distinctiveness</td>
<td></td>
</tr>
<tr>
<td>distinctive</td>
<td>52.2%</td>
</tr>
<tr>
<td>very distinctive</td>
<td>47.8%</td>
</tr>
<tr>
<td>Age</td>
<td></td>
</tr>
<tr>
<td>adults</td>
<td>95.3%</td>
</tr>
<tr>
<td>sub-adults</td>
<td>4.1%</td>
</tr>
<tr>
<td>immatures</td>
<td>0.6%</td>
</tr>
</tbody>
</table>
Photographic analysis

Based on 32 groups, a mean proportion of 0.51 individuals (SD = 0.24, range: 0.17-1) were WM and 0.78 (SD = 0.16, range: 0.42-1) were at least slightly distinctive. Proportions of marked individuals within groups composed exclusively of residents, visitors or transients, differed between groups (Table 3). The inference tests showed that the proportions of marked individuals in groups composed exclusively of transients were significantly higher than those from groups composed exclusively of residents, when either considering WM or slightly distinctive individuals. Twelve groups including individuals of different residency patterns (mixed groups) were excluded (Table 3).

Of the 103 WM-individuals with recaptures, 29 (28.2%) incurred new notches in the dorsal fin. Of these, 17 underwent multiple mark changes. Mark changes were recorded on 43 occasions, for a total of 52 new notches. Considering that multiple mark changes between recaptures could have occurred on either a single or multiple occasions, the actual number of mark change events was somewhere between 42 and 53. The cumulative time between the first and last capture for all individuals captured more than once was 125737 days (343.5 years), thus a new mark event was calculated to occur every 6.6-8.0 years (mean = 7.3 years).

Pilot whales were captured in every calendar month. The mean group size was 18 individuals (median = 15, SD = 12, range: 2-60, n = 105). Larger groups were recorded between May-October and were composed of individuals of different residency patterns (Figure 6A-B), however, the two-way ANOVA test revealed no significant differences in group size between months (P = 0.58), between type of groups (P = 0.09), or due to the interaction of both factors (P = 0.84). Groups dominated by resident individuals were naturally recorded year-round. Groups dominated by transients were also recorded year-round (although masked in the second semester by being recorded in mixed groups). Finally, groups dominated by visitors, as well as mixed groups, were proportionally more frequent between August-December (Figure 7). The two-sample proportions test showed that the proportion of mixed groups (compared to other types of groups) was significantly higher (P < 0.001) for the period July-December than for January-June. Despite the non-inclusion of the effort analysis in Figure 7, the probability of encountering mixed groups or another type of groups was the same within each month. The different number of encounters used in each month does not reflect seasonal abundances. A spatio-temporal analysis taking effort into consideration is in preparation and will be published elsewhere. A closer examination of capture histories revealed that mixed groups included all types of possible associations between individuals of different residency patterns.

Table 3. Mean proportions (±SD, range) of well-marked and of at least slightly distinctive individuals from groups composed exclusively of residents (R), of visitors (V), and of transients (T); and P-values for the one-way ANOVA and post-hoc Tukey tests (* 0.01 < P < 0.05). n - number of samples

<table>
<thead>
<tr>
<th></th>
<th>Well-marked individuals</th>
<th>At least slightly distinctive individuals</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Residents</td>
<td>0.35 (±0.13, 0.22-0.50)</td>
<td>0.68 (±0.13, 0.56-0.83)</td>
<td>5</td>
</tr>
<tr>
<td>Visitors</td>
<td>0.49 (±0.16, 0.33-0.67)</td>
<td>0.79 (±0.11, 0.67-1)</td>
<td>7</td>
</tr>
<tr>
<td>Transients</td>
<td>0.66 (±0.22, 0.25-1)</td>
<td>0.89 (±0.11, 0.71-1)</td>
<td>8</td>
</tr>
<tr>
<td><strong>all groups</strong></td>
<td>0.51 (±0.24, 0.16-1)</td>
<td>0.78 (±0.16, 0.42-1)</td>
<td>32</td>
</tr>
<tr>
<td>one-way Anova</td>
<td>P = 0.0259*</td>
<td>P = 0.0159*</td>
<td></td>
</tr>
<tr>
<td>post-hoc Tukey</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R - T</td>
<td>P = 0.0222*</td>
<td>P = 0.0125*</td>
<td></td>
</tr>
<tr>
<td>R - V</td>
<td>P = 0.3983</td>
<td>P = 0.2601</td>
<td></td>
</tr>
<tr>
<td>V - T</td>
<td>P = 0.2096</td>
<td>P = 0.2229</td>
<td></td>
</tr>
</tbody>
</table>
The social network diagram, based on 344 individuals from 246 encounters, shows that 124 individuals (36%) form a core social network and the remaining 220 individuals (64%) form satellite clusters (Figure 8). The diagram comprises 39 (11.4%) residents, 72 (20.9%) visitors and 233 (67.7%) transients. While the main component includes all the resident individuals, but also visitors and transients, the 46 satellite clusters do not include any resident and were dominated by clusters of transients only (84.8%). Additionally, the satellite clusters include clusters of visitors only (6.5%) as well as clusters with both transients and visitors (8.7%).

The SD and CV of the observed pairwise association indices were significantly higher than those from permuted data sets, when using either the simple ratio or the half-weight index \( (P < 0.001) \), thus rejecting the null hypothesis that individuals associated randomly. The cluster dendrogram (Figure 9, CCC = 0.96) shows that most of the whales captured on ≥4 encounters were captured with preferred companions, and one pair was always captured together (ID numbers 310 and 493 from pod V3, Table 4). The mean of the half-weight association index per dyad was 0.77. The estimate of social differentiation was 1.87 (SE = 0.16). A total of 44 individuals from 66 encounters with high- or full-coverage between 2005-2011 were used for this analysis, 33 (75.0%) residents and 11 (25.0%) visitors.

When assessing population division into social entities, the modularity-G suggests that the best division is with an association index of 0.181, and the knot-diagram suggests a knot at an association index of 0.471 (Figure 9). The modularity-G peak (0.608) is high, showing that with this division, there is much more total association within pods than would be expected for randomly determined pods. The modularity-G was the selected method for pod division due to its high maximum value and due to the subjective interpretative nature of the knot-diagram. Therefore, a total of eight pods of variable size and association strength were identified,

Associations

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five being composed by resident individuals (R1-5) and three by visitors (V1-3). Exceptions occurred in pod R2 where one visitor individual is included, and in pod V3 where one resident individual is included (Figure 9, Table 4). A mean pod size of 15 individuals (SD = 9, range: 4-29) was estimated including marked and non-marked individuals by applying the previously estimated correction factors of the proportion of well-marked individuals to resident and visitor pods (Table 4). A one-way ANOVA revealed no significant differences ($P = 0.38$) between the pod and group mean sizes.

A total of 90 individuals comprising residents, visitors and transients captured during 66 encounters (corresponding to 53 days between 2005-2011) were used to address temporal pattern in social relationships between all individuals in the study area. The SLAR line never reached the null association rate, indicating the absence of random associations for the time scale of ≈ 2000 days. The line started to decline only after about three years (Figure 10). The best fit model also indicates a social system with long-lasting relationships. Therefore, the fitted model described as ‘casual acquaintances’ is not appropriate in this case (Table 1). To address temporal pattern in social relationships between individuals of a resident pod the analysis was restricted to the nine individuals from pod R4 (the pod with most captures). The social system model that best fit the curve is described by the model including the constant companion hypothesis over this period (Figure 1ESM).

**Genetic analysis**

Among the 29 distinct samples successfully analysed, 12 were females and 17 were males. Females and males of all three residency patterns were recorded (Table 1ESM). All short and all long sequences were identical. The haplotype corresponding to the short sequences perfectly matched the haplotype corresponding to the long sequences (GenBank accession numbers JX144881 and JX144880, respectively). Thus, haplotype diversity was null. Microsatellite allelic diversity ranged between 4 and 11 (Table 5). Average expected heterozygosity ranged between 0.423 and 0.831. No loci departed from HWE. Loci were not in linkage disequilibrium, except for FCB1 and FCB17 ($P < 0.001$). The probability of genotype identity was $3.8 \times 10^{-7}$, and $2.6 \times 10^{-3}$ for first order relatives.

Genetic differentiation between individuals identified as resident ($n = 13$) and transient ($n = 6$) was not significant ($F_{ST} = 0.015, P = 0.181$; $R_{ST} = -0.020; P = 0.611$). As for relatedness, mean $r_{Q&G}$ was 0.004 (SE = 0.238) among individuals.
identified as resident, -0.054 (SE = 0.307) among those identified as transient and -0.037 (SE = 0.246) in the entire dataset. Related individuals ($r_{Q\&G} > 0.1$) tended to be over-represented within groups (Figure 11). Noticeably, individuals with $r_{Q\&G} > 0.4$ represented 12.1% of intra-group pairwise comparisons, while between groups their proportion was only 1.9%. In the entire dataset, there were three pairs of individuals with $r_{Q\&G} > 0.5$, which could be considered as potential first order relatives. A resident adult female (ID 116) and a resident subadult male that were sampled in the same group had $r_{Q\&G} = 0.745$. They shared one allele in common for each microsatellite locus, indicating that they could be mother and offspring. There were also a resident female and a transient male with $r_{Q\&G} = 0.500$ and a resident male and a male of unknown residency pattern with $r_{Q\&G} = 0.574$, which had not been sampled in the same groups. These individuals could not be parent and offspring as they did not share one allele in common at every loci.

Mean $r_{Q\&G}$ within groups (mean = 0.097, SE = 0.265) was higher than between groups (mean = -0.016, SE = 0.237). The Mantel test revealed a significant correlation between genetic relatedness and group membership (Pearson $r = 0.161$, $P = 0.003$). Mean intra-group relatedness was negatively correlated to group size (Spearman’s rank coefficient, $n = 8$, $r = -0.807$, $P < 0.05$; Figure 12). Mean relatedness was high (around 0.3) in groups of up to 30 individuals, and low (below 0)

Figure 9. Top: dendrogram produced using average-linkage hierarchical cluster analysis (CCC = 0.96) for 44 well-marked pilot whales based on high-quality images captured on ≥ 4 encounters with high- or full-coverage. Sampling period as well as associations was defined as individuals grouped within an encounter. Eight pods composed either by residents (R) or by visitors (V) were identified, with exceptions in pods R2 and V3 where one visitor (*) and one resident (+) individuals (as defined in Methods) were included, respectively (see individuals’ attributes in Table 4). Bottom left: the knot-diagram suggests a knot at an association index of 0.471 (dashed line). Bottom right: the modularity-G suggests that the best division into pods is with an association index of 0.181 (dotted line).
in groups of 45-50 individuals. In a group of 50 resident individuals where six samples were obtained, average relatedness was low, but two subgroups with a mean relatedness of 0.148 (first two samples) and 0.068 (last four samples) could be defined. In a group of 45 resident individuals where
three samples were obtained, the first sample that was obtained was unrelated to the last two, while the latter two presented a mean relatedness of 0.251. Finally, there was a significant correlation between pairwise relatedness coefficients and half-weight indexes for the 12 individuals that had been biopsied and photo-identified (Mantel test: Pearson $r = 0.380$, $P = 0.002$).

**DISCUSSION**

This study shows that pilot whales demonstrate a large degree of variability in site fidelity, including residents, regular visitors and transients. These differences in patterns of occurrence were not due to a sampling bias since whales from the three types of residency pattern were recorded over the entire course of the study and all were highly distinctive, thus having similar probabilities of being recaptured. The fact that some resident individuals have $>30$ recaptures, and that one resident individual had a 14-year recapture interval, suggests that some short-finned pilot whales exhibit long-term site fidelity in Madeiran waters. Nevertheless, the range of their distribution is unknown, and our findings do not imply that they were always present in the study area. The fact that the visitors regularly visit (and not just pass by) Madeira suggests that these animals come to these waters for either mating and/or feeding. Transients, on the other hand, were captured throughout the entire course of the study and year-round and this suggests that they belong to an open pelagic population that is far from being fully catalogued, and they use the Madeiran waters as a ‘passage’. The analysis of the social network diagram shows that these whales associate with visitors and residents during their passage. Independently of the importance of the Madeiran waters for these whales, they constituted about $\frac{2}{3}$ of our catalogue. Heimlich-Boran (1993) also reported whales captured only once in the neighbouring archipelago of the Canary Islands. The extent of this ‘meta-population’ is unknown, and it is likely that some of these whales visit both archipelagos. As suggested by Heimlich-Boran, perhaps they simply return at intervals greater than the length of the study. In fact, in the present study, two whales catalogued as transients had a 10- and a 9-year recapture interval; which may comply with the previous suggestion. Varying patterns of residency have been also commonly documented among cetacean populations (Silva et al., 2008; Baird et al., 2009), however, for pilot whales, only Mahaffy (2012) had described a pattern of habitat usage similar to ours.

The results from mark rate support the occurrence different communities. When comparing the proportion of marked individuals between groups composed exclusively of residents, visitors or transients, groups of transients have more distinctive individuals than groups of residents ($P < 0.05$). Not discarding bias potentially associated with the analysis, individuals of all ages and both sexes had the same capture probability between each residency pattern, thus natural differences can in fact occur. The reason why transients may be more likely to bear dorsal fin notches is beyond the scope of this study. To the best of our knowledge, this is the first study testing the distinctiveness of individuals according to residency pattern, and this question should be addressed in future photo-id studies where groups of different residency patterns co-occur.
Our results suggest that, despite the existence of three residency patterns in pilot whales around Madeira, resident whales are not isolated from visitors and transients. Mixed groups of individuals with different residency status were frequently observed between July and December, and the social network diagram shows that whales of different residency patterns were photographed together. The genetic analyses did not reveal any differentiation between resident and transient whales, neither for mitochondrial-DNA nor for nuclear-DNA. These analyses are preliminary given the low number of biopsied whales with known residency pattern. Lack of genetic differentiation will have to be confirmed by the analysis of a higher number of individuals of the three residency patterns. It is noteworthy that mitochondrial-DNA haplotype diversity was null. Although short-finned pilot whales are known to have very low mtDNA diversity, which is likely to result from their matrilinear social structure (Whitehead, 1998), this complete lack of diversity was unexpected. It suggests a common ancestry for all biopsied whales, independent of their residency pattern.

The results from group size analysis also support the idea that we are facing one community in the islands, and others that move about and breed when they meet. As observed in various studies performed year-round in other areas, large groups tend to occur at a specific season of the year (e.g. Felleman et al., 1991; Cañadas and Sagarminaga, 2000; de Stephanis et al., 2008). Heimlich-Boran (1993) reported that larger groups of short-finned pilot whales occurred during the summer months in the Canary Islands. His findings are in accordance with ours suggesting that, in the warm-temperate waters of the north-east Atlantic, larger groups of this species occur in the warmer months of the year. The reason for such aggregations could be linked to foraging behaviour, as evidenced for this species and Risso’s dolphins (Grampus griseus) off California (Shane, 1995). However, there is not sufficient information about prey availability to infer a similar association for pilot whales in Madeira, nor for the Canary Islands (Heimlich-Boran, 1993). An alternative and non-exclusive hypothesis is that whale aggregations are linked to breeding behaviour. The season of larger group size corresponds to the likely mating period observed in the Canary Islands (Heimlich-Boran, 1993). Similarly, in Madeira, the observation of larger groups coincides with an increase of visitors and a statistically significant higher proportion of mixed groups during the warmer months, making it possible for individuals of different residency patterns to breed. Furthermore, in a matrilineal species, increasing group sizes is believed to occur mainly for reproduction, as suggested for aggregations of long-finned pilot whales (de Stephanis, 2007). Recently, satellite tagging of different clans of pilot whales in the Mediterranean Sea also supported the hypothesis that clans interact for mating purposes when they find each other (Verborgh et al., 2012). Finally, our estimated mean pod size (15, SD = 9, range: 4-29, n = 8) was lower (but not statistically significant) than the mean group size (18, SD = 12, range: 2-60, n = 105). While the low number of pods analysed limits the interpretation, this discrepancy could arise from temporary associations between pods. The means of pod and group sizes obtained in this study are in agreement with observations made in other pilot whale populations (Heimlich-Boran, 1993; Ottensmeyer and Whitehead, 2003; de Stephanis et al., 2008; Mahaffy, 2012).

This study shows that pilot whales in Madeira exhibit a well-differentiated society with long-lasting relationships. The results of the social analysis rejected the null hypothesis that individuals associated randomly and the dendrogram revealed that most of the whales captured on ≥4 encounters were with preferred companions. The temporal analysis also indicated an absence of random associations for the time scale of ≈ 2000 days (six years). Additionally, despite the SLAR decline, a detailed examination of the capture histories reveal that some resident individuals have been frequently captured together during the six years period considered for the temporal analysis, as corroborated by the SLAR of the pod R4 that fit a constant companion model, with no disassociations over that period. The SLAR decline after a stabilization of approximately two-three years indicates some gradual disassociations over that period, which may indicate a demographic event where individuals are dying or leaving the identified population (Whitehead, 2008). Another explanation is that, when a newborn arrives, the relationship between a mother and a previous calf decreases with time. Considering that the weaning period in this species ranges between 2.5 and 5.5 years (Kasuya and Marsh, 1984), it could have contributed to the decline in the SLAR. The positive correlation between association indexes and genetic
relatedness coefficients suggested that individuals who associated more tended to be genetically related. This preliminary result would be worth investigating by further analyses.

Genetic relatedness was higher within groups than between groups and highly related individuals tended to be over-represented within groups as compared to between groups. Mean intra-group relatedness was negatively correlated to group size. Although mean relatedness is naturally expected to decline with group size due to a dilution effect, the existence of a correlation implies that small groups are made up of related individuals. Interestingly, genetically related subgroups could be defined within two of the largest groups, which suggest that large groups may be temporary associations of two or more unrelated groups. Similarly, a genetic study on striped dolphins in the Ligurian Sea showed that average relatedness significantly decreased with group size, and concluded that large groups were aggregations of smaller social units (Gaspari et al., 2007). Also in that study, mean relatedness within groups was higher than between groups, especially in females where \( r_{Q\&G} \) reached a mean value of 0.221.

Our results indicate that the pods outlined here represent stable social entities and suggest that short-finned pilot whales may exhibit natal group philopatry, a type of stable matrilineal social structure implying relatedness between members of both sexes within the natal group, as described in killer whales in the coastal eastern North Pacific (Bigg et al., 1990; Barrett-Lennard, 2000). The number of samples available for the genetic analyses was too small to compare relatedness between genders. Mean relatedness coefficients obtained for resident as well as for transient individuals were low, and much lower than in populations of killer whales exhibiting philopatry in both sexes, where mean \( r_{Q\&G} \) ranged between 0.102 in transient and 0.305 in resident populations (Pilot et al., 2010). Nonetheless, our study tends to corroborate the hypothesis described by Heimlich-Boran (1993), that the short-finned pilot whale has a matrilineal hierarchical system. The temporal analysis, fitting a constant companion model to a resident pod, also supports the hypothesis that we are facing a matrilineal system. These findings, together with the evidence that long-finned pilot whales also exhibit a matrilineal structure (Amos et al., 1991a, b, 1993a, b; Fullard, 2000) support the idea that *Globicephala* spp. follow this social pattern.

The final goal of this study is to discuss the implications of our findings for the long-term conservation of this species and the possible management measures that could be implemented. If we had found several populations, or at least one of island-associated/residents and other(s) of oceanic/transients, those would require a high level of concern, with intensive monitoring and possible severe conservation measures, given that population differentiation would prevent gene flow and not enable the island-associated/residents to recover from dramatic events. Instead, the results suggest potential gene flow between different types of communities, which would prevent genetic divergence of island-associated communities. Therefore, there might be a single population in the warm-temperate oceanic waters of the north-east Atlantic. From a conservation standpoint, this ‘meta-population’ can be regarded as a single management unit, whose preservation can be of extreme importance as it may act as a pool for resident communities. Similar studies should be conducted in the neighbouring archipelagoes of the Canary Islands and Azores to determine whether there is a need for concerted conservation policies at the scale of the North Atlantic. On the other hand, the presence of island-associated whales, whether residents or regular visitors, imply a high risk of exposure to local threats, which can also result in implications for the whole population. Even if we consider that different communities should not be regarded as demographically independent populations, these island-associated whales should be considered as stable social entities in governmental management plans. Therefore, an assessment of the potential threats should be carried out, as well as a periodic evaluation of the status of these social entities. Due to the growing whale-watching industry, proper legislation and/or educational workshops with the operators are also recommended, given that it could minimize the impact over those entities.

To conclude, we would like to raise an often debated but still unsolved question; what is a social entity, or a conservation unit? The social entities observed in the present study were defined as ‘pods’. However, the biological significance of pods has been questioned. The term ‘pod’ that has been used by Bigg et al. (1990) to describe groups of closely related matriline of killer whales that travel, forage, socialize and rest with each other ≥50% of the time, and are stable over many...
generations, has been later redefined by Ford and Ellis (2002) as transitional groupings that reflect the relatedness of recently diverged matrilines. The clusters that we and Heimlich-Boran (1993) interpreted as meaningful pods are likely the closest equivalent (since it satisfies the criterion of being associated ≥50% of their time) to ‘units’ in other studies characterizing stable social entities of pilot whales (Otensmeyer and Whitehead, 2003; de Stephanis et al., 2008; Mahaffy, 2012). Following the suggestion that the social structure of pilot whales is based on clans (i.e. pods which associate regularly; de Stephanis, 2007; de Stephanis et al., 2008), the results from the hierarchical cluster analysis suggest possibly three clans of island-associated whales, one made by pods V1, V2 and R1, another by R2, R3 and R4, and a possible third one by R5 and V3.

It is then proposed that the pilot whales encountered in Madeira belong to a single population encompassing several clans, possibly three clans of island-associated whales and others of transients, each containing two to three matrilineal pods, each with a mean of 15 individuals (SD = 9, range: 4-29). Given the difficulty in identifying these pods at sea, we suggest that all clans, whether composed of island-associated or of transient whales, should be treated equally as a precautionary approach. Finally, further research in this species is encouraged due to the high proportion of marked individuals and low rate of mark change obtained in our study.

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