Cultural transmission of tool use combined with habitat specializations leads to fine-scale genetic structure in bottlenose dolphins

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Socially learned behaviours leading to genetic population structure have rarely been described outside humans. Here, we provide evidence of fine-scale genetic structure that has probably arisen based on socially transmitted behaviours in bottlenose dolphins (Tursiops sp.) in western Shark Bay, Western Australia. We argue that vertical social transmission in different habitats has led to significant geographical genetic structure of mitochondrial DNA (mtDNA) haplotypes. Dolphins with mtDNA haplotypes E or F are found predominantly in deep (more than 10 m) channel habitat, while dolphins with a third haplotype (H) are found predominantly in shallow habitat (less than 10 m), indicating a strong haplotype–habitat correlation. Some dolphins in the deep habitat engage in a foraging strategy using tools. These ‘sponging’ dolphins are members of one matriline, carrying haplotype E. This pattern is consistent with what had been demonstrated previously at another research site in Shark Bay, where vertical social transmission of sponging had been shown using multiple lines of evidence. Using an individual-based model, we found support that in western Shark Bay, socially transmitted specializations may have led to the observed genetic structure. The reported genetic structure appears to present an example of cultural hitchhiking of mtDNA haplotypes on socially transmitted foraging strategies, suggesting that, as in humans, genetic structure can be shaped through cultural transmission.

1. Introduction

Darwinian selection acts on phenotypes, which are manifested through both genetically and non-genetically inherited traits. Both inheritance mechanisms may be adaptive and, thus, of evolutionary consequence [1]. Vertical social transmission (i.e. learning from a biological parent) closely follows genetic inheritance patterns. For example, the diets and/or foraging strategies of offspring have been shown to resemble that of their mother in a wide range of mammalian taxa (e.g. orangutans, Pongo pygmaeus wurmbii; sea otters, Enhydra lutris nereis; and bottlenose dolphins, Tursiops sp.) [2–4]. All three species share prolonged maternal dependence and there is overlap between nursing and offspring-foraging during development, providing opportunities for social learning [2,3,5].

Social transmission can affect the evolutionary outcomes of genetic transmission and vice versa. Social transmission may change selection pressure on genes (gene-culture coevolutionary theory [6]), as for example documented for the spread of lactose tolerance in adult humans [7] and proposed for hundreds of human genes [8]. Further, individual learning capacity or the exposure to a socially learned trait can correlate with specific genetic marker systems [6].
For instance, in vertical social transmission, patterns of transmission of the socially learned trait and uniparentally inherited genetic units, such as mitochondrial DNA (mtDNA) and the Y chromosome, may be closely correlated. If the socially learned trait increases its bearer’s fitness, population frequencies of these correlated genetic units will increase, even if there is no other active selection on genetic units. This phenomenon is called ‘cultural hitchhiking’ [9] and was posed as a possible explanation for the low genetic diversity in human mtDNA and Y chromosome sequences [10] and the low mtDNA diversity in matrilineral whales [9]. However, stochastic modelling has shown that a reduction of genetic diversity is not a necessary consequence of parallel transmission of genes and socially learned phenotypes [10,11].

Cultural hitchhiking might occur with little or no fitness differences if there is fine-scale geographical population structure that relates to the cultural trait, as we discuss below. There are two general types of geographical population structure: continuous clines or sharp boundaries [12]. Genetic and geographical distances often correlate in natural populations (i.e. isolation-by-distance, e.g. [13]). On the other hand, geographical features resulting in habitat fragmentation or behavioural patterns limiting movement and genetic exchange may lead to discontinuous genetic structuring of populations. There is ample evidence for structuring by geographical boundaries [14]. However, there is limited evidence for geographical structuring of genetic variation due to behaviour and even less evidence due to cultural behaviour. Possible mechanisms include assortative mating and micro-habitat specialization. For example, two species of cichlid fish (Amphilophus xiloaensis and Amphilophus sagitae) preferentially mate with a partner of the same colour morph, which has led to genetic differentiation between morphs [15]. Moreover, in killer whales (Orcinus orca), socially transmitted foraging specializations within an ecosystem were proposed to have led to sympatric ecotypes in the absence of physical barriers [16,17].

To date, studies describing the influence of social transmission or culture on genetic structure or selection have focused on genetic variation between populations [8]. Here, we provide an example of how social transmission may drive genetic structure within a single dolphin population.

Thirteen foraging strategies have been described for bottlenose dolphins in the eastern gulf of Shark Bay (ESB), Western Australia [3]. Individual females have been observed engaging in one to seven of these strategies [3], some of which are only observed in specific habitats. The most prominent foraging strategies observed only in shallow water include beach hunting, bottom grubbing and kerplunking [18,19]. Foraging strategies observed in deep water include ‘sponging’ [20–23]. The question of whether sponging is a socially transmitted behaviour has been studied extensively in Shark Bay in the past [3,20,21,24–28]. These studies used different approaches (i.e. genetics, individual follows, survey data, network-based approaches, individual-based modelling or combinations thereof) to infer social transmission of tool use in this population.

Sponging dolphins (‘spongers’ hereafter) carry conical marine sponges on their rostrums, most likely to protect them while foraging for prey hiding in the substrate [21,27]. The behaviour is almost exclusively vertically transmitted from mothers to their offspring through social learning [3,20,24]. With one exception, spongers in ESB share a maternally inherited mtDNA haplotype [24]. Spongers in ESB are also biparentally more closely related than expected by chance [24]. Haplotypic sharing between sponging individuals is expected in cases of strong vertical social transmission [24], even though a genetic basis for sponging has been ruled out [24,25,28]. Sponging has also been documented in the western gulf of Shark Bay (WSB) [23,25,29]. The ESB and WSB study sites are approximately 120 km apart by sea, divided by a prominent peninsula, and there is no direct evidence of dispersal between the sites [30]. In both gulfs, sponging is almost exclusively limited to deep channels where sponges occur [22,23]. However, not all individuals inhabiting deep channels use sponges to forage. Therefore, a purely ecological explanation for sponging would not account for the heterogeneity of foraging strategies observed in these deep channels [22,24].

This study focuses on the fine-scale population genetic structure of bottlenose dolphins in WSB. We investigated the relationship between maternal relatedness, habitat and behaviour. Our study significantly extends previous studies in that it explores the possibility of social transmission shaping genetic structure in an animal population. This is a very important advance because, to our knowledge, this study provides the first example of the potential impact of social learning on within-population structure in non-human animals (as opposed to its influence on between-population structure [15–17]). Briefly, we observed striking spatial homogeneity in mtDNA in contrast to other parts of the bay, where haplotype distribution was much more heterogeneous [30]. We aimed to test the hypothesis that vertical social transmission of a habitat-dependent trait can lead to fine-scale genetic structure. To do this, we modelled the possible process using an individual-based model based on empirical data. We used this model to investigate whether the observed homogeneous mtDNA haplotype structure could be a result of a random process or, alternatively, would require that transmission patterns of a socially learned trait and uniparentally inherited genetic units correlate, as is already known in this case [3,20,21,24–28]. The model showed that the observed geographical structuring of mtDNA haplotypes is only possible if genetic and social transmissions are correlated.

2. Material and methods

(a) Study site and data collection

Shark Bay is located on the west coast of Australia, 850 km north of Perth. It consists of an eastern and western gulf, divided by Peron Peninsula. Our main study area is off the township of Useless Loop in the western gulf and consists of approximately 260 km², about five times the size of the average home range of an adult ESB bottlenose dolphin [31]. We will refer to our main study area as WSB. WSB is characterized by channels (more than 10 m deep) dividing shallow water areas that are closer to land (figure 1) [23].

We carried out systematic photo-identification and behavioural surveys on bottlenose dolphins from a 5.4 m boat with an outboard motor. Dolphin group composition (10 m chain rule [32]), water depth, GPS and predominant dolphin activity (forage, rest, travel, social or unknown) were recorded. For our analyses, we did not discriminate between these activities. Dolphins were individually identified by natural markings and the shape of their dorsal fin [33]. Biopsy sampling [34] was conducted under a Licence to use and/or supply Animals for Scientific Purposes from the Western Australian Department of Environment and Conservation, and
ethics approval was obtained from the University of New South Wales (08/338), Murdoch University and the University of Zurich.

A sponger was defined as a dolphin that was seen sponging at least twice [20]. We were able to use water depth as a proxy for habitat because Tyne et al. [23] reported that sponges only occur in water deeper than 10 m, while seagrass occurs in shallow water (less than 10 m) in WSB.

(b) Analyses

The genetic sampling and laboratory methods are detailed in the electronic supplementary material. We ran a nested ANOVA in PASW STATISTICS 18 to test for significance of association between water depth distribution of dolphins with different mtDNA haplotypes. Because multiple sightings of the same individuals were included, individual dolphin identification (ID) was used as a random factor. To exclude the possibility that the observed mtDNA haplotype distribution might simply be an artefact of significant autosomal population structure, we tested whether the observed segregation of mtDNA haplotypes and depth is reflected in biparentally inherited microsatellite markers. Such autosomal structure would have to be very strong, causing a very clear mitochondrial geographical structuring. To investigate this, we ran a STRUCTURE analysis (burn-in length of 105 and 106 Markov chain Monte Carlo steps) using mtDNA haplootypes and 106 Markov chain Monte Carlo steps) using mtDNA haplootypes as a basis for randomization reflected the frequencies calculated for the WSB dolphins. The randomizations were carried out in a ‘macro’ written in Microsoft Excel.

To test whether spongers are biparentally more related than expected by chance, we calculated the average pairwise relatedness among spongers and compared it to the average pairwise relatedness of the population. We calculated the Queller & Goodnight [39] estimator of pairwise relatedness (R) in SPAGEDi [40]. In order to assess statistical significance, we programmed a randomization test in MATLAB R2010a (MathWorks, Natick, MA, USA). Biparental genotypes of all sampled individuals of the population were randomized and the average pairwise relatedness among 22 or 15 (for WSB and ESB, respectively, representing the number of biopsy sampled spongers) randomly drawn genotypes were calculated. In the randomization, the observed sex ratio of spongers was retained.

(c) Individual-based model

We used simulations to test the hypothesis that vertical social transmission of a habitat-dependent trait can lead to fine-scale genetic structure using an individual-based model based on empirical data. The model is described in detail in the electronic supplementary material. We included simulations of a null model with no fitness benefits for specialist, as well as one with fitness benefits. Most simulations were run with 5% fitness benefits for specialists, even though empirical data suggest that fitness advantages of spongers may be larger than that, albeit being non-significant [20].

3. Results

(a) Mitochondrial DNA haplotypes and habitat

We found a strong and significant association between dolphin mtDNA haplotypes and water depth in WSB. Dolphins with mtDNA haplotypes E or F were predominantly found in deep water (more than 10 m deep) and dolphins with haplotype H in shallow water (less than 10 m, figures 1 and 2).
The geographical segregation of mtDNA haplotypes in WSB was not reflected in biparentally inherited genetic markers: STRUCTURE plots showed no indication of genetic structure (electronic supplementary material, figure S2). The depth distribution for both adult males and females with one of the three common haplotypes E, F and H differed significantly (tables 1 and 2), as revealed by a nested ANOVA and a subsequent Tukey’s HSD post hoc test, based on 90 dolphins (59 females and 31 males) and 756 sightings (mean number of sightings + s.e./individual: 8.4 + 0.68, range = 1–33). Three other haplotypes (B, D and I) were rare (frequency less than 0.03).

(b) Genetic relatedness of spongers
Forty spongers have been identified in WSB, which represents about a quarter (25.9%) of dolphins identified in deep water [29]. All 22 biopsied spongers in WSB share the same mtDNA haplotype E, which is significantly different (p < 0.001) from what would be expected by chance, based on the observed mtDNA haplotype frequencies in the population. The sponger haplotype E in WSB is different from the haplotype found among ESB spongers (haplotype H [24]). All 22 sampled spongers were biparentally more closely related than the population average (Rspongers = 0.0259, Rpopulation = -0.0104, Npopulation = 108), although this difference was not statistically significant (p = 0.092). We also re-ran the pairwise relatedness analyses for ESB [24], including additionally genotyped microsatellite loci and individuals. Our result confirms the previous publication [24]: spongers in ESB were found to be biparentally more closely related than the population average and than expected by chance (Rspongers = 0.0712, Rpopulation = -0.0044, Nspongers = 15, Npopulation = 238, p = 0.006).

(c) Individual-based model
Simulations showed that fine-scale genetic structure based on mtDNA haplotypes can be driven by vertically, socially transmitted habitat-dependent traits (figure 3; electronic supplementary material, figure S3). The geographical segregation of haplotypes occurred even if the learning fidelity was not 100% (electronic supplementary material, figure S3), but in the absence of vertically, socially transmitted specializations, no geographical segregation of mtDNA haplotypes was observed (figure 3, fourth column).

4. Discussion
Through a combination of empirical data and individual-based modelling, we were able to infer that the vertical cultural transmission of a foraging behaviour involving tools has led to the clear separation of mtDNA haplotypes within our study area. The observed geographical distribution of dolphins with different mtDNA haplotypes might represent a case of cultural hitchhiking. To our knowledge, this is one of the first studies to provide evidence for fine-scale geographical genetic structure driven by socially transmitted behaviour within a single wild animal population.

In what circumstances would we predict correlations between habitats and mtDNA haplotypes? We propose that four prerequisites must be met. First, a population must exhibit vertically socially transmitted, habitat-dependent skills, such as foraging or predator avoidance strategies. Second, philopatry must keep haplotypes localized, although if one sex disperses, the pattern could still be found in the philopatric sex. Third,
Figure 3. mtDNA haplotype segregation by habitat in an individual-based model. Three mtDNA haplotypes (hap.1, 2 and 3) and two habitat specializations (sponging and strategy2) were present. All spongers had hap.1 and all strategy2 individuals had hap.2. Top row: number of different females per strategy (no. IDs/strategy). Bottom row: proportion of individuals with a particular mtDNA haplotype in deep water relative to all individuals with this particular mtDNA haplotype. Proportions were calculated for every haplotype separately. Error bars represent 1 s.e. Dashed lines indicate the observed haplotype proportion in deep water for the three mtDNA haplotypes E, F and H in WSB. Fitness benefits for specialists are shown below graphs. Because random cultural drift [41] is a strong force counteracting the establishment of new innovations, we indicated the likelihood (%) of at least one specialist/strategy to persist for 100 time periods; this is shown below the fitness benefits. Learning fidelities equalled 1 for daughters born to specialists for the simulations shown here. Simulation results with various other learning fidelities and fitness benefits are shown in the electronic supplementary material, figure S3. In the first column, individuals of both strategies had the same fitness benefits. In the second column, only one strategy (sponging) was present, in the third column, strategy2 was innovated 50 time periods after sponging and had a higher fitness, and in the fourth column, there were no specializations present.

### Table 1: mtDNA Haplotype Proportions and Fitness Benefits

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>Fitness</th>
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<tbody>
<tr>
<td>hap.1 (sponger)</td>
<td>1.05</td>
</tr>
<tr>
<td>hap.2 (strategy2)</td>
<td>1.05</td>
</tr>
<tr>
<td>hap.3</td>
<td>38.0%</td>
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</tbody>
</table>

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<tr>
<th>Haplotype</th>
<th>Fitness</th>
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<tbody>
<tr>
<td>sponger</td>
<td>1.05</td>
</tr>
<tr>
<td>strategy2</td>
<td>n.a.</td>
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<th>Haplotype</th>
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<tbody>
<tr>
<td>hap.1 (sponger)</td>
<td>1.05</td>
</tr>
<tr>
<td>hap.2 (strategy2)</td>
<td>1.1</td>
</tr>
<tr>
<td>hap.3</td>
<td>18.1%</td>
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</table>

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>Fitness</th>
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<tbody>
<tr>
<td>sponger</td>
<td>n.a.</td>
</tr>
<tr>
<td>strategy2</td>
<td>100%</td>
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Habitat differentiation needs to be on a scale that is larger than an individual’s home range. Fourth, in order for habitat specializations to drive genetic differentiation over small distances, these specializations must be stable over an individual’s lifetime and also present in following generations. The clear separation of matriline and habitats we present here suggests that habitat specializations have been in place for many more generations than, for instance, the four generations of spongers that have been observed in ESB so far.

The segregation of mtDNA haplotypes by habitat appears to be driven by vertically transmitted foraging specializations (and/or other behaviours leading to habitat specialization). The absence of fine-scale genetic structure based on biparentally inherited markers shows that the correlation of maternally inherited mtDNA haplotype and water depth cannot be explained solely by geographical separation. Of course, it is possible that there is undetected autosomal structure, but it is most unlikely that this would be strong enough to secondarily cause the extremely sharp mtDNA patterning that we observed. That any autosomal structuring within our study area must be only weak is supported by two points. First, our use of locprior ensures detection of relatively weak structure [37]. Second, the relatively weak autosomal genetic differentiation between ESB and WSB was documented using a much smaller number of markers [30] than used in this study.

Different depth preferences exhibited by dolphins with different mtDNA haplotypes coincide with different habitats. Seagrass meadows are characteristic of shallow water, and conical sponges only grow in water deeper than 10 m in WSB [23]. Shallow and deep habitats intergrade and are not divided by a barrier or distance that could prevent dolphins from moving between deep and shallow water. The absence of genetic structure based on biparental genetic markers indicates that there is no mating barrier between deep and shallow habitat and may be explained by the dispersal patterns of Shark Bay dolphins. Both sexes are philopatric, with males expanding their natal range [30,31]. In the WSB study site, we observed the dolphins foraging, travelling, resting and socializing. Therefore, the geographical segregation of dolphins with different mtDNA haplotypes indicates that dolphins stay in their natal habitat for all those activities (we do not know about mating). Male bottlenose dolphins in Shark Bay cooperate with one another in pairs and trios to consort females [43]. It appears that allied males can direct a female outside her regular home range. Some sightings of females with haplotype H in deep water or haplotype E in shallow water might be explained by the coercion of males. It is possible that these groupings involved consorting of females by males; however, we did not confirm the consortships by the standards of ESB [43]. In ESB, males have been observed consorting females never seen in the study site before and well-studied females altered their depth-use during consortships [44].

Although our study site is not likely to encompass the entire home ranges of some individuals [45] included in this study, it is about five times the size of an average adult...
ESB dolphin home range [31]. Accordingly, we expect our identification of each individual’s habitat usage to be suitably representative. In addition, the number of sightings per individual (up to 33) suggests that the study site covers a great portion of the home ranges of at least some animals.

Our individual-based simulations emphasize that social transmission of habitat-dependent specializations can lead to fine-scale genetic structure (figure 3, first, second and third columns); whereas this fine-scale structure is unlikely to be seen if there is no social transmission (figure 3, fourth column). Social transmission of foraging strategies in WSB appears likely, given the mtDNA haplotype sharing among WSB spongers, as occurs in ESB [24]. Our finding that spongers in WSB are not more closely related than expected by chance provides further evidence that sponging is transmitted socially, rather than genetically, and in a vertical fashion. Furthermore, our simulations indicate that two specializations are necessary, one for the deep and one for the shallow habitat, in order to obtain the observed segregation of two mtDNA haplotypes by habitat. It is conceivable that traits other than sponging are vertically socially transmitted, such that matrilines H and F also exhibit habitat specialization. Without any habitat specialization, no mtDNA haplotype segregation is observed (figure 3, fourth column).

Although all spongers sampled in WSB belong to one matriline, it is a different matriline from that of spongers in ESB [24]. The presence of different sponging matrilines in each gulf can be explained most parsimoniously by the occurrence of at least two independent innovations, one in each gulf. Alternatively, there could have been horizontal transmission between matrilines. However, this would have to be extremely rare to fit the patterns that we observe.

What we have inferred for sponging may be just one example of a more widespread phenomenon of interactions between culture and geographical structure of genetic variation. Genetic differentiation on relatively small scales has been reported for many bottlenose dolphin populations (both Tursiops aduncus or Tursiops truncatus) in all oceans. This structuring was attributed largely to habitat specialization and was found based on maternal and biparentally inherited markers [46–50]. However, most of these studies (except [46]) gave no indication of what the specializations might be, or how they could have driven the genetic structure. Furthermore, the geographical scales at which genetic differentiation have been documented (tens to hundreds of km) in previous studies are much greater than those reported here: deep and shallow habitats in WSB are separated by just tens of metres. The largest delphinid, the killer whale, may present a similar case. Sympatric ecotypes were shown to differ genetically and in their foraging behaviour [16,51]. The genetic differentiation between ecotypes based on microsatellites suggests that this separation may be more stringent and/or has been in place for longer than that in WSB.

A correlation between habitat and genetic structure has also been found in tool-using New Caledonian crows (Corvus moneduloides) [52]. Rutz et al. [52] found biparental and maternal (e.g. mtDNA haplotypes) genetic structure among three habitats (dry forest, farmland and beachside habitat, less than 10 km apart), in which different tool use has been observed. Restricted dispersal may lead to localized occurrence of particular tool use [52]. In female mountain gorillas (Gorilla beringei beringei) and western gorillas (Gorilla gorilla diehli), Guschnsksi et al. [53] reported a correlation between microsatellite-based genetic and geographical structure (i.e. altitude). This correlation did not hold for males. In gorillas, both sexes disperse, but females usually join a neighbouring group. Hence, female dispersal distance is limited and often occurs within habitat types. The authors concluded that dispersal usually results in females staying within the habitat into which they were born, probably owing to food preferences [53]. Unfortunately, data on maternally inherited markers was not presented. The correlation between habitat and mitochondrial genetic structure in bottlenose dolphins reported here holds for both adult females and males.

If vertical social transmission influences the fine-scale genetic structure of a population (e.g. within WSB), it may have consequences for the genetic structure at larger scales (e.g. between the two gulfs of Shark Bay). As WSB matrilines seem to stay in their natal habitats, it is likely that they have behaviourally adapted to those habitats. If male and female dolphins in Shark Bay are philopatric because of learnt foraging strategies, genetic differentiation would occur—as observed—over small geographical distances. Limited dispersal for both sexes could potentially cause inbreeding. Indeed, elevated levels of inbreeding were measured in ESB, but the costs may not be high enough to outweigh the benefits of philopatry [54]. Philopatry bears benefits of habitat familiarity and the potential for kin cooperation [55]. This study highlights that cultural forces can shape genetic population structure outside humans and on a small geographical scale.

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Data accessibility. Genotypes used in this study have been archived in the Dryad Digital Repository (doi:10.5061/dryad.k5b1j).

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