

Supplementary Information: Multi-Network-Based Diffusion Analysis reveals vertical cultural transmission of sponge tool use within dolphin matriline

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Genetic methods

Biopsied individuals were genotyped based on 27 microsatellite markers (Tab. S1), following the protocols detailed in [1]. We obtained the allele frequency for each locus (Tab. S1; Tab. S2), as well as missing and error rates (Tab. S1) from empirical data, and used them as input parameters for simulations in COANCESTRY [2]. Error rates for each locus were based on 29 individuals that had been genotyped more than once (Tab. S1). To select the best performing relatedness estimator, we simulated 1,000 genotypes based on the empirical allele frequency in the population (Tab. S2). Subsequently, we simulated 100 dyads each for half siblings (relatedness (r) = 0.25), parent-offspring (r = 0.5), full siblings (r = 0.5), first cousins (r = 0.125) and unrelated individuals (r = 0). The estimator TrioML was chosen as the most accurate estimator, showing lowest variance and highest correlation with the true data (Tab. S3; Tab. S4). We then calculated dyadic biparental relatedness among individuals using TrioML [3].

Table S1: Microsatellite markers used to assign genotypes, including error and missing rates, for bottlenose dolphins in the western gulf of Shark Bay, Western Australia.

Locus	Missing rate	Error rate	Reference
E12	0.003	0.000	[4]
MK6	0.003	0.000	[5]
Tur4_105	0.000	0.000	[4]
Tur4_108	0.000	0.000	[4]
Tur4_111	0.017	0.000	[4]
Tur4_117	0.000	0.034	[4]
Tur4_128	0.007	0.000	[4]
Tur4_66	0.000	0.000	[4]
Tur4_98	0.000	0.000	[4]
D22	0.003	0.000	[6]
D8	0.047	0.000	[4]
F10	0.007	0.000	[4]
Tur4_138	0.000	0.000	[4]
Tur4_141	0.000	0.000	[4]
Tur4_87	0.000	0.034	[4]
Tur4_91	0.000	0.000	[4]
Tur4_162	0.000	0.000	[4]
MK9	0.007	0.000	[5]
MK5	0.000	0.000	[5]
Tur4_132	0.000	0.000	[4]
KWM12	0.000	0.000	[7]
EV37	0.041	0.000	[8]
Tur4_80	0.000	0.000	[4]
MK3	0.007	0.000	[5]
Tur4_142	0.000	0.034	[4]
Tur4_153	0.000	0.000	[4]
MK8	0.007	0.000	[5]

Table S2: Allele frequencies for 27 microsatellite markers in the bottlenose dolphin population of the western gulf of Shark Bay, Western Australia.

Marker																	
E12	Alleles	260	276	280	264	272	256										
	Frq	0.1854	0.2007	0.051	0.1412	0.3793	0.0425										
MK6	Alleles	154	166	156	174	182	184	172	160	176	152	188	168	180	190	186	
	Frq	0.0969	0.0289	0.1156	0.0969	0.017	0.0714	0.1497	0.0697	0.1259	0.1803	0.0374	0.0034	0.0017	0.0017	0.0034	
Tur4_105	Alleles	391	367	395	399	387	403										
	Frq	0.1746	0.5458	0.1153	0.0915	0.039	0.0339										
Tur4_108	Alleles	270	258														
	Frq	0.7271	0.2729														
Tur4_111	Alleles	299	303	307	287	295											
	Frq	0.1052	0.7776	0.0672	0.0448	0.0052											
Tur4_117	Alleles	183	179	187	175												
	Frq	0.5915	0.3288	0.0661	0.0136												
Tur4_128	Alleles	303	307	295	299	311											
	Frq	0.5973	0.2031	0.1229	0.0751	0.0017											
Tur4_66	Alleles	201	193	197	205	189											
	Frq	0.7932	0.1644	0.0102	0.0237	0.0085											
Tur4_98	Alleles	192	196														
	Frq	0.4051	0.5949														
D22	Alleles	116	118	110	120												
	Frq	0.4031	0.4677	0.0731	0.0561												
D8	Alleles	326	342	322													
	Frq	0.4751	0.306	0.2189													
F10	Alleles	386	390	382	378												
	Frq	0.3549	0.07	0.43	0.1451												
Tur4_138	Alleles	223	215	207	219	227	211										
	Frq	0.2746	0.4102	0.0644	0.0915	0.1576	0.0017										
Tur4_141	Alleles	238	250	242	282	246	278	254	234	230	218						

	Frq	0.0576	0.2746	0.2542	0.0458	0.1288	0.0254	0.1119	0.061	0.0136	0.0271						
Tur4_87	Alleles	186	178	190	182	194											
	Frq	0.6492	0.1305	0.178	0.0237	0.0186											
Tur4_91	Alleles	227	207	223	231	235	215	211	219								
	Frq	0.3932	0.239	0.0373	0.2085	0.0373	0.0254	0.0237	0.0356								
Tur4_162	Alleles	407	411	403													
	Frq	0.3542	0.5797	0.0661													
MK9	Alleles	168	174	172	170	176	178										
	Frq	0.4352	0.1177	0.2133	0.2116	0.0205	0.0017										
MK5	Alleles	205	213	211	215	219											
	Frq	0.1288	0.3356	0.2949	0.2322	0.0085											
Tur4_132	Alleles	330	334	326													
	Frq	0.9136	0.078	0.0085													
KWM12	Alleles	166	170	174	186	156	164	168	182	190	184	160	188	178	162	161	
	Frq	0.3576	0.1729	0.1763	0.0881	0.0712	0.0153	0.0136	0.039	0.0034	0.0475	0.0017	0.0017	0.0017	0.0085	0.0017	
EV37	Alleles	204	210	216	202	220	212	194	222	218	224	206	208				
	Frq	0.3339	0.3145	0.0901	0.0495	0.0389	0.0689	0.0477	0.03	0.0159	0.0053	0.0018	0.0035				
Tur4_80	Alleles	311	323	291	315	319	303	327									
	Frq	0.0458	0.3356	0.1305	0.2525	0.1831	0.0305	0.022									
MK3	Alleles	161	163	157	165	167	147	169									
	Frq	0.099	0.3567	0.0956	0.3891	0.0256	0.0034	0.0307									
Tur4_142	Alleles	330	342	334	338												
	Frq	0.1458	0.0492	0.1559	0.6492												
Tur4_153	Alleles	215	219														
	Frq	0.7271	0.2729														
Tur4_MK8	Alleles	103	109	111	107	113	105	87	115	97							
	Frq	0.1451	0.0939	0.4471	0.244	0.0119	0.0222	0.0256	0.0068	0.0034							

Table S3: Summary statistics of seven relatedness estimators resulting from simulations in COANCESTRY

n=600	TrioML	Wang	LynchLi	LynchRd	Ritland	QuellerGt	DyadML	TrueValue
Mean	0.267	0.268	0.265	0.269	0.278	0.262	0.289	0.271
Variance	0.038	0.046	0.047	0.052	0.095	0.046	0.039	0.033
MSE	0.010	0.015	0.015	0.019	0.060	0.015	0.011	

Table S4: Correlation matrix of seven relatedness estimators and the simulated true value

Correlation Coef	TrioML	Wang	LynchLi	LynchRd	Ritland	QuellerGt	DyadML	TrueValue
TrioML	1							
Wang	0.927	1						
LynchLi	0.927	0.969	1					
LynchRd	0.884	0.834	0.841	1				
Ritland	0.666	0.595	0.610	0.820	1			
QuellerGt	0.923	0.930	0.962	0.844	0.635	1		
DyadML	0.99	0.938	0.936	0.894	0.669	0.932	1	
TrueValue	0.860	0.820	0.822	0.790	0.610	0.821	0.859	1

Maternity analyses

To test for influences of vertical social learning on sponging, we created a network reflecting the mother-offspring relationship based on field observations of 278 mother-offspring pairs. To match additional mother-calf pairs, we ran maternity analyses in CERVUS 3.0.7 [9] for individuals with haplotype E and H (since 42 spongers with known haplotype carried haplotype E, and one sponger carried haplotype H) and with no more than three microsatellite loci missing. (Note that the single individual carrying haplotype H is a male sponger, who was only seen with a sponge twice out of 25 observations. He is not a regular sponger, but rather appears that have picked up sponges that other individuals have dropped without necessarily using them as foraging tools, as has been observed in other individuals (unpublished data). We nevertheless decided to include him as a sponger in the analysis to be consistent with our definition of a ‘sponging’ individual [see manuscript]).

First, in order to obtain critical values of likelihood ratios used for parentage analysis on empirical data, a maternity simulation was run for individuals with haplotypes E and H separately. As the mitochondrial haplotype is maternally inherited, candidate mother and offspring always carry the same haplotype. To determine the number of candidate mothers for all offspring in the E and H data set, respectively, we created a home range with all GPS locations of observations of individuals carrying haplotypes E and H, respectively (for details on calculating home ranges, see below). We then calculated home range overlap of all individuals who were either female or of unknown sex (*i.e.*, excluding genetically known males) and who had any overlap with the E or H home range, respectively. This resulted in 355 candidate mothers for the haplotype E data set and 343 candidate mothers for

the H data set, both of which are likely a conservative over-estimate (since some individuals of unknown sex will be male). Other input parameters were set as following: the proportion of sampled individuals (calculated as the proportion of sampled females out of the total number of candidate mothers) was set to 0.44 (for both E and H), while the allele frequency (Tab. S2), the proportion of loci typed (= 0.995) and the proportion of loci mistyped (= 0.0038) were obtained from empirical data – the latter stemming from 29 individuals who had been genotyped more than once. The output of the simulations (run for 1,000 offspring) was then used to run the maternity analyses on empirical data.

To match mother-offspring pairs reliably, we only considered matches with ‘logarithm of the odds’ (LOD) scores significantly higher (at 5% level) than the critical value obtained from simulations [9] and with dyadic relatedness estimates between 0.426 and 0.631, which corresponded to the range of relatedness estimates between known mother-offspring pairs. Furthermore, we only matched mother-offspring pairs where approximate birth date of both candidates (based on speckle levels [10], body size, and time of first offspring for females) was known and at least 10 years apart [11].

In the network modelling vertical social transmission, we set entries between all mother and offspring pairs to 1, as dyadic association strength - which has been used in the network modelling horizontal and oblique learning (see main text) - would not capture vertical learning opportunities in cases where offspring have been weaned and no longer associate with their mothers. Therefore, entries of 1 better reflect our knowledge that mother and offspring have spent several years in close association, during which vertical learning could have occurred.

Calculation of home range overlaps

Diffusion of a foraging skill, like sponging, might follow an association network simply because individuals who spend a lot of time together also experience the same ecological conditions. Being subject to the same ecological conditions, individuals might therefore tend to learn the same foraging skills asocially. If this were the case, we would expect a network of similarity in habitat usage to be a better predictor of the pattern of diffusion, since individuals who do not spend time together but utilise the same environments would be similarly predisposed to learn the skill. Therefore, unless environmental usage and the social network are highly correlated, one could distinguish between these alternatives and/or quantify the relative influence of each. We used dyadic home range overlap as a proxy for the extent to which two individuals experience the same ecological conditions. For each individual with at least seven sightings, a home range was defined using 95% Epanechnikov kernel density estimates (R package `adehabitatHR` [12]). When calculating kernel densities, the choice of a smoothing factor greatly influences the accuracy of the estimated home range and should thus be

carefully considered [13]. The commonly used smoothing factor *href* (reference bandwidth), which is defined as

$$href = \sigma * n^{-1/6}$$

$$\text{where } \sigma = 0.5(\sigma_x + \sigma_y)$$

assumes that the true distribution of observations follows a normal distribution [14]. If this assumption is violated, *href* tends to over-smooth and therefore overestimate home ranges [15]. Nevertheless, it is often preferred over alternative methods, such as least-square cross validation (LSCV), which makes no assumption about the true distribution but tends to under-smooth and cannot be estimated in many cases [12]. Visual inspection, which can aid in finding an appropriate smoothing factor [13], of 12 different dolphin home ranges revealed that *href* tended to over-smooth home ranges, particularly in cases where relocations were far apart, but under-smooth home ranges with only a few relocations that were close together. We therefore selected a subjective smoothing parameter by setting a lower limit of 1,000 and an upper limit of 4,000 for *href*, and then calculated a new smoothing factor for each individual as

$$h = 0.5(href) + 1,500$$

$$\text{where } 1,000 \leq href \leq 4,000,$$

which appeared to more accurately reflect the 12 inspected home ranges, given the number and distribution of the sightings (Fig. 1).

In order to remove land from the estimated kernel densities (the land boundaries in our study area were too complex to implement the ‘boundary’ parameter provided in *adehabitatHR* [12]), each individual’s utilisation distribution was multiplied with a grid (100 m resolution) with values of 1 (for grid cells on water) and 0 (for grid cells on land). We then re-weighted each grid cell within an individual’s home range to ensure that, overall, the kernel density added up to 1 again [16]. We then calculated dyadic home range overlap (95%) using the ‘utilisation distribution overlap index’ (UDOI) (*adehabitatHR* [12]), which is considered most accurate when quantifying space-use sharing [17].

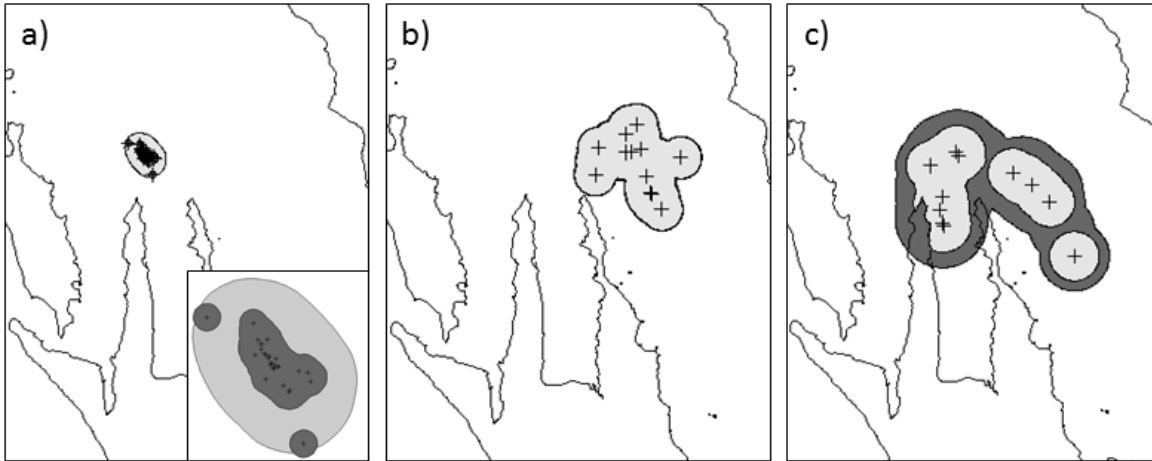


Figure 1: Contours (95%) of home ranges with reference bandwidth (*href*; dark grey) and customised smoothing factor after land removal (light grey) for a) a home range with small smoothing factor (*href*: 632; *custom*: 2,000; 29 sightings); b) a home range with an average smoothing factor (*href*: 3,181; *custom*: 3,090; 12 sightings); and c) a home range with large smoothing factor (*href*: 5,703; *custom*: 3,500; 11 sightings).

Selecting a threshold for including individuals to maximise the power of NBDA

Since NBDA infers social learning if a behaviour follows the social network, there is a trade-off between sample size and data quality. Only considering individuals above a certain threshold of sightings (*i.e.*, dropping individuals with few sightings) can increase certainty about the strengths of connections within the network but, at the same time, decrease the power of NBDA to reliably detect social learning, especially if linking individuals are removed [18]. To select a threshold that maximises the power of NBDA to detect social learning, we ran a simulation approach [18] – for computational reasons only considering individuals that had been seen at least five times ($N = 538$ individuals). A social learning process is simulated though the population assuming learning follows the NBDA model [19]. The resulting order of acquisition is then used in an NBDA which uses a social network with introduced observational error that varies with the number of times each dyad has been seen together or apart. Low numbers of sightings may result in greater error, while a large number of sightings results in a value that is closer to the real strength of association between individuals. The power of NBDA is then calculated as the percentage of models where social learning correctly outperforms asocial models. We ran the simulations with parameters $s = 20$ (selected through trial and error) and number of learners = 9, and tested for cut-off points of 5-20 sightings (while dropping all individuals below the cut-off point regardless of whether they were informed or not). The social learning parameter s estimates the strength of social transmission per unit of association with informed individuals relative to the rate of asocial learning [19]. The number of individuals that learned spicing between 2007 and 2018 in our empirical data set was 18. However, maternity data was unavailable for nine individuals and they were thus excluded from the NBDA analysis as learners (see manuscript), which

is why we set the number of learners in the simulation to nine instead of 18. A threshold of seven sightings resulted in highest statistical power, with an acceptable (though slightly conservative) level of a false positive error rate (1.2%), *i.e.*, when s was set to 0. The simulation approach is set up to simulate learning among associated individuals and does not take different pathways into account. Therefore, the threshold of seven maximises the power of NBDA to detect horizontal social learning in the sponging data set.

Applying NBDA

Since exact dates of the acquisition of sponging were not known, we applied the ‘order-of acquisition diffusion analysis’ (OADA) [19]. In OADA, social learning is inferred if the order with which individuals learn the behaviour follows the social network. Unlike the alternative ‘time of acquisition diffusion analysis’ (TADA), OADA does not make any assumptions about the baseline rate of acquisition, which may have fluctuated over time as changing prey availability and environmental conditions made sponging more or less likely to be learned, across the population.

Nine spongers with no maternity data available were removed from the diffusion using the `filteredNBDAdata` function provided in the NBDA package v0.6.1 [20].

Previous studies using NBDA with the inclusion of individual-level variables (ILVs) have selected between an ‘additive’ model, in which the ILVs affect only the rate of asocial learning, and a ‘multiplicative’ model, in which the ILVs all affect both asocial learning and social transmission in the same way. Here, we used an approach suggested by [21] and fit a more general ‘unconstrained’ model, in which the effects of each ILV on asocial and social learning are estimated as independent parameters. Thus, we allow for the fact that i) some variables might influence social learning without forcing the model to assume that all variables do so; and ii) variables might have a different effect on asocial and social learning.

We found that standard errors for transmission parameters s and for the ILVs could not be reliably obtained, because of highly asymmetrical profile likelihood. This also makes standard errors a misleading measure of precision. Therefore, we derived 95% confidence intervals for parameters using profile likelihood techniques [22] based on the best predictive model.

Influence of ILVs on social and asocial learning rates

Results suggested an increase in vertical social learning of sponging when being female. None of the other ILVs were associated with the learning rate of sponging (socially or asocially) (Tab. S5).

Table S5: Support for ILVs and model average estimates for ILVs with relative support >0.5.

ILV	Sex (females to males)	Average water depth	Average group size	Haplotype
Relative support for effect on social learning	0.975	0.167	0.110	0.069
Model averaged estimate * (back-transformed)	126	-	-	-
Profile likelihood confidence interval conditional on the best model	[9.5; 2897]	-	-	-
Relative support for effect on asocial learning	0.056	0.017	0.060	0.056
Model averaged estimate	-	-	-	-
Profile likelihood confidence interval conditional on the best model	-	-	-	-

*Model averaged estimates are weighted medians across the set of values. In OADA, extreme values can badly skew weighted means, even in models with a very small model weighting. Thus, we used medians as a more robust estimate.

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