

Report on killer whale population genetics for the BRT review on the status of the southern resident population

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Introduction

The killer whale (*Orcinus orca*) is a widespread species known to have strong matrifocal social structure in some populations (see Bigg et al. 1990). Worldwide census data are few, and there are no reliable estimates of total abundance. A recent survey has estimated 70,000 in the Antarctic (Dahlheim & Hening 1999). The population size, and especially its stability over time, together with reproductive behavior (especially reproductive skew) will in part determine the effective population size for this species. Relatively low population density suggests relatively low effective population size, and this will be reflected in genetic diversity. However, the worldwide levels of genetic diversity at mitochondrial DNA (mtDNA) loci are low even in the context of low effective population size (Hoelzel et al. 2002, LeDuc unpublished). Furthermore, data from tests for neutrality and the pattern of diversity in the mtDNA control region contributed to the conclusion by Hoelzel et al (2002) that this species had been through a population bottleneck, and that the lack of diversity resulted from this historical event. The implication is that some of the diversity seen in either worldwide or regional context may reflect remnant diversity that pre-dates the stochastic loss of diversity during the bottleneck. This would mean that the magnitude of differences between regional haplogroups may not reflect time in isolation.

The strong matrifocal structure of some regional populations has further implications for population genetic structure. The level of mtDNA diversity in these local populations will reflect the level of female philopatry and their time in isolation. Given time, local matrilineal lines will acquire variation through mutation. Hoelzel et al. (2002) describe a fixed (apparently the same in all individuals of a given population) mtDNA difference between the southern resident and Southeast Alaskan resident

populations in the eastern North Pacific (ENP). However, the magnitude of this difference was just 1bp in 1,826bp (0.05%). This fact, together with the uniformity of haplotypes in each population suggested that there was strict female philopatry, that the local ‘populations’ represented matrilineal clans, and that the two resident clans were separated quite recently in evolutionary terms (Hoelzel et al. 2002). The transient population found from California through Southeast Alaska was also apparently fixed for one mtDNA haplotype, which differed from the southern resident haplotype at 8bp (0.44%), still a small genetic distance, though suggesting a more ancient divergence than the difference between the resident haplotypes (or remnant diversity). In each case these data provided information only on the dispersion of females. Preliminary data suggested the possibility of greater male-mediated gene flow (Hoelzel et al. 2002, Barrett-Leonard unpublished) based on relatively low and uniform genetic distance among ENP populations (especially in the context of maternal philopatry, high within population kinship and consequent low effective population size).

In a commissioned study, I undertook to extend the mtDNA data to incorporate further population samples from the ENP, and to investigate nuclear genetic diversity for a large number of loci (17) to provide better resolution on the question of male-mediated gene flow. This is the subject of this report.

Materials and Methods

Sample collection

Samples were collected from stranded, captive and free-ranges whales (the latter by biopsy sampling, see Hoelzel et al. 1998). Regions from which samples were collected are shown in Figure 1. Sampling strategy for the Alaskan resident (AR) and southern resident (SR) putative populations (only) included females that represented known, extended matrilineal lines. In this way we could infer the genotype of un-sampled individuals (see Hoelzel et al. 2002). Further details on sample sizes are indicated below, but note that all sample sizes indicated reflect only the analysed samples, and not additional individuals for whom mitochondrial genotypes can be inferred. The total sample-set also included some pairs of whales known to be related along a matriline, and

only one individual from these pairs were sequenced for mtDNA. DNA was extracted by standard phenol-chloroform methods.

The sample set for microsatellite DNA analyses included a total of 320 samples, some of which were repeat extractions of the same sample, or repeat samples from the same whale. These were included as internal controls. A total of approximately 300 genotypes (among the 17 loci) were repeats incorporated into the analysis to help ensure the accurate calling of alleles. This included repeats on both the same and different gels. Among the samples available, only a subset could be definitively assigned to a putative population (as some were from captive or stranded animals), and some were from regions where very few samples were available (insufficient to represent a population). Therefore, this report will focus on the 211 samples that could be assigned reliably to populations. These can be broken down as follows: 30 from the southern residents, 40 from the Southeast Alaska residents, 14 from a putative 'resident' population off Kamchatka in Russia (all have the southern resident mtDNA type), 20 from a putative 'resident' population off the Aleutians and in the Bering Sea (all have the northern resident mtDNA type), 10 from the 'offshore' population, 35 from transient pods sampled in southeast Alaska, 22 from transient pods sampled in California (all of these transient pods had the same mtDNA haplotype), and 40 samples collected from the southeast region of Iceland.

PCR amplification

Primers for mtDNA situated in tRNA_{thr} and tRNA_{phe} amplify the tRNA_{pro} and entire control region loci (N=188, including 133 ENP, 45 ENA, 5 WSA, 3 WSP, 1 WNA, & 1 ANT, see Figure 1; including data for 102 sequences from Hoelzel et al. 2002), for a combined total of 995bp (for primers and reaction conditions see Hoelzel & Green 1998). No variation was found in the tRNA_{pro} region, and therefore this amplification product is referred to as 'control region'. Amplified DNA was purified on QIAGEN spin columns and sequenced forward and reverse using the ABI 377 automated system. Microsatellite DNA was amplified from 17 loci. The references and PCR protocols are provided in Table 1. PCR conditions required extensive titration for this species. Amplified microsatellite DNA was analyzed for length variation on 6% polyacrilamide denaturing

gels using fluorescent imaging on an automated ABI PRISM 377 DNA sequencer, after incorporation of 1/10 fluorescent labelled primer. An internal standard marker (Genescan-500 ROX, Applied Biosystems) was used to determine the allele sizes.

Data analysis

Haplotypes were aligned using pileup (GCG computer package) or CLUSTAL X and compared using un-rooted neighbor-joining phylogeny reconstructions (using PAUP* 4.2 and MEGA). The transition/ transversion ratio was set at observed levels. Bootstrap analyses were run for 1,000 replications. The calculation of nucleotide diversity was after Nei (1987) and computed using ARLEQUIN version 2.0 (Schneider et al, 1999). F_{ST} (using the formulations described by Weir and Cockerham 1984) and the significance of its difference from zero were calculated using FSTAT (Goudet 2001). The correlation between genetic and geographic distance was tested for a subset of the populations using a Mantel test as implemented in GENEPOP 3.1d (Raymond & Rousset 1995a,b). Fu's F_s (Fu 1997) was estimated using ARLEQUIN version 2.0.

The level of genetic diversity was estimated as observed heterozygosity (H_o), expected heterozygosity (H_e) and allelic richness. Allelic richness controls for variation in sample size by a rarefaction method, and was calculated using the program FSTAT 2.9.3 (Goudet 2001). Genotyping is incomplete for one or two populations for three loci, therefore allelic richness data is provided only for the other 14 loci. Evaluation of possible deviations from Hardy Weinberg (overall deviation, heterozygote deficiency and heterozygote excess) was performed using Fisher's exact test and the Markov chain method (dememorization number, number of batches, iteration per batch set at 1,000, Bonferroni correction applied) using ARLEQUIN. Linkage disequilibrium was tested using Fisher's exact test and the Markov chain method (dememorization number, number of batches, iteration per batch set at 1,000, Bonferroni correction applied), implemented in FSTAT.

The most probable number of putative populations (K) that best explains the pattern of genetic variability was estimated using the program STRUCTURE 1.0 (Pritchard *et al.*, 2000). We assumed the admixture model and performed the analysis considering both the independent and the correlated allele frequency model. Burning length and length of

simulation were set at 500,000 and 1,000,000 repetitions, respectively. To test the convergence of the priors and the appropriateness of the chosen burn in length and simulation length, we ran a series of independent runs (4 repeats) for each value of K (for $1 < K < 9$) as suggested by Pritchard *et al.* (2000). We tested whether any particular individual was an immigrant or had an immigrant ancestor by using the model with prior population information, subdividing the individuals into K populations, according to the results of the previous analysis. We assumed ν (migration rate) = 0.05 and 0.1, and testing for $0 < \text{number of generations } (G) > 1$.

An asymmetric estimate of the migration rate ($M=4N_e m$) between pairwise populations, based on microsatellite data, was calculated using MIGRATE (Beerli, 2002) for a subset of the populations. The lengths of the runs were optimised for both markers (acceptance-rejection $> 2\%$, $R < 1.2$). Initial runs were set estimating θ and M with F_{ST} and allowing M to be asymmetric. Reruns were set using the parameter estimated found with the first run and lengthening the MCMC chains. For comparison the migration rate was also calculated based on private alleles using GENEPOP 3.1d. This method provides a multilocus estimate of the effective number of migrants (Nm) according to Slatkin (1985). A corrected estimate is given using the values from the closest regression line (see Barton and Slatkin, 1986).

Results

mtDNA diversity

Among the 188 control region sequences, 16 haplotypes were defined by 21 variable sites (Figure 2). The samples used in this analysis came from across the world (Figure 1), though the North Pacific is much better represented than other regions (see methods). The overall level of diversity was low at $\pi = 0.004$, comparable to levels seen in species known to have undergone a bottleneck (e.g. $\pi = 0.004$ for the northern elephant seal; Hoelzel *et al.* 1993). The new data from the North Pacific (including samples from the Aleutians, Bering Sea and Kamchatka Peninsula) provided three further haplotypes within the transient lineage, all closely related to the previously published haplotypes

(Figure 2; Hoelzel et al. 2002). There are three fixed differences between the transient lineage (composed of five haplotypes) and the resident lineage (composed of two haplotypes), which could reflect either time in isolation, or chance differences between remnant haplotypes that pre-date a population bottleneck. Tests to assess the pattern of diversity and evidence for population expansion were consistent with the results described in Hoelzel et al. (2002). An unrouted neighbor joining phylogeny still showed a star-like structure (Figure 3), and a linearised version of the tree shows how shallow the diversity is worldwide (Figure 4). This tree further illustrates three putative lineages that may reflect haplogroups that have evolved from post-bottleneck haplotype remnants. These are also represented in the alignment figure (Figure 2) through the delineation of two of the putative lineages (boxes A&B).

We can estimate the time since a putative bottleneck by assessing the accumulated variation within lineages. Considering only transversions, the average number of changes per lineage is 0.67 (range = 0-1; excluding ANT where the sample size is 1). Based on estimates of the transversion mutation rate from interspecific comparisons (Hoelzel et al. 1991, Barnes et al. 1985), this would suggest a bottleneck event approximately 145,000 to 210,000 YBP (based on *O. orca* v *Cephalorhynchus commersonii*: 23 transversions; estimated 5 MY divergence time; and *O. orca* and *C. commersonii* v *Balaenoptera acutorostrata*: 95 transversions (ave); estimated 30MY divergence time). However, given the incomplete and uneven representation of haplotypes within lineages, and the small number of transversions, this will be an approximate estimate (see Hoelzel et al. 2002 for further discussion). The possibility of a post-bottleneck population expansion is supported by Fu's F_s statistic (-8.29, $p < 0.001$).

Population structure based on microsatellite DNA diversity

Diversity was measured among and within the eight putative populations (see methods) for 17 microsatellite DNA loci (see Table 1). The level of heterozygosity and allelic diversity is provided in Tables 2&3, including data on the significance of any differences between expected and observed heterozygosity. There was no significant linkage disequilibrium among any of the locus pairs.

Measures of F_{st} comparing the eight putative populations are shown in Table 4. The significance of the difference from zero for each value is given (without Bonferonni correction). A Mantel test was used to test the relationship between genetic and geographic distance for the four putative ‘resident’ populations (southern residents, Southeast Alaskan residents, resident haplotype whales from the Aleutians/ Bering Sea region, and resident haplotype whales from the Kamchatka Peninsula region in Russia; Figure 5). A Spearman’s rank correlation test was significant at the $p=0.07$ level. Geographic distances were approximate, as the precise delineation of these population ranges is unknown.

The assessment of population structure based on Bayesian likelihood estimates (using STRUCTURE) is given in Figure 6. The highest likelihood was found for $K = 7$ populations, and the likelihood value was flat among the four replicate runs. All putative populations were supported with the exception of the ‘Bering Sea’ population, which appeared to be composed of a mixture of animals from Kamchatka and Southeast Alaska, perhaps indicating a population boundary in that region. Note that all of these animals have the northern resident mtDNA haplotype, as do the Southeast Alaskan residents, while the Kamchatka animals have the southern resident mtDNA haplotype. After assigning 7 populations as indicated by the initial runs, we could identify several putative migrants. These are indicated in Figure 7 with arrows. Only those with a significance of $p < 0.001$ are shown. One of these suggests a migration event between the Offshore and Transient communities (between the two main mtDNA lineages in the North Pacific).

Further assessment of gene flow was undertaken using both the private allele method and the coalescent method used in MIGRATE. All pairwise comparisons for the estimates based on private alleles are shown in the upper diagonal of the matrix in Table 4. The comparisons are generally consistent with ‘ecotype’ (transient vs resident type) and geographic distance. Only a subset of populations have been compared using MIGRATE to date, but this work is still in progress. The results for the three populations compared using MIGRATE are given in Table 5. All geneflow estimates reflect either ongoing geneflow or historical geneflow. The distinction will be assessed by further analyses in future.

Discussion

The mitochondrial DNA data presented here are consistent with the previous study suggesting a population bottleneck (Hoelzel et al. 2002). The evidence for population expansion (F_s) remained significant, and the structure of the mtDNA control region now shows an even stronger effect of unexpectedly high variation in the central conserved domain. The further samples analyzed identified a lineage of haplotypes representing transient populations in Alaskan waters and in the Bering Sea. Some of these haplotypes have been described elsewhere (Barrett-Leonard, unpublished; LeDuc, unpublished). Transient pods from southern California through Southeast Alaska still showed just one haplotype, as did the additional southern resident and Southeast Alaskan resident pods sampled. New populations sampled in the Bering Sea, along the Aleutians, and off Kamchatka in Russia extended the known distribution of the ENPNR and ENPSR haplotypes (see Figure 2). The southern resident haplotype (ENPSR) has now been identified from animals sampled in Russia, the Aleutians, Puget Sound, off Newfoundland, and off the coast of England. It is the most widely distributed mtDNA haplotype so far recorded for the killer whale. There was no correlation between phylogenetic lineages and foraging ecotype (mammal vs fish predation), and only limited consistency with geographic populations.

The fact that local populations of 'resident' pods in coastal habitat throughout the North Pacific are composed of individuals all fixed for the same mtDNA haplotype, suggests that these represent local founder events, perhaps initially founded by single, matrifocal pods. Without further immigration from other matrifocal groups, these populations could expand, generating new pods by fission, and retain the same mtDNA haplotype among all individuals in the extended clan. The level of microsatellite DNA diversity also appears to be relatively low for these coastal resident populations (Tables 2&3; especially in comparison with transient pods), which would be consistent with this theory (though the rigorous assessment of relative levels of diversity at these markers will require further analysis). The very shallow differentiation between the two resident haplotypes (0.05%) suggests recent coancestry, and perhaps historical occurrence in a common population prior to the founding of the extant coastal populations. The founding

may have occurred after the habitat became available after the last glacial epoch. The distribution of the same closely related haplotypes among populations across this wide geographic range indicates that the haplotypes diverged in a common source population prior to the founding of the coastal populations. Although we still know little about behavior in transient pods, they appear to be more fluid in composition, and transients seem to find resources over a broader geographic range. These factors may lead to greater outcrossing, and consequently greater genetic diversity.

As an initial assessment of the pattern of diversity among putative populations, using genetic markers that reflect the movement of both males and females, eight populations were defined on the basis of both behavior and geographic distribution (southeast Iceland, southern residents, Southeast Alaskan residents, Bering Sea residents, Russian residents, California transients, Southeast Alaskan transients, and offshores). The assessment of the offshore group needs to be taken as very preliminary due to the small sample size and its likely heterogeneity, given a broad geographic range for the inclusion of samples (from southern California to Alaska). These putative populations were compared at 17 microsatellite DNA loci. A likelihood method that identifies populations based on equilibrium expectations with respect to Hardy-Weinberg and linkage using only individual genotypes (implemented in STRUCTURE) supported seven of the eight putative population designations. The 'Bering Sea' group appeared to be a mixture of Russian resident and Southeast Alaskan resident animals (see Figure 6). All eight populations were never-the-less included in tests for population structure and geneflow.

Measures of the interpopulation inbreeding coefficient (F_{st}) indicated that about 3% - 25% of the variance could be explained by differences among putative populations, depending on the comparison. These values are comparable in magnitude to population differences in other species of large mammals (e.g. Kermode bears in British Columbia: Marshall & Ritland 2002; lynx in Scandinavia: Rueness et al. 2003; Wolves in Europe: Lucchini et al. 2004). The smallest difference was between the Bering Sea and Southeast Alaskan residents, and the largest differences were between the Icelandic and North Pacific populations. Estimates of geneflow based on private alleles were very consistent with the pattern of differentiation shown by the F_{st} data (Table 4). The data indicate that

both geographic distance and behavior led to the differentiation of populations. Further, among putative populations of a behavioral type (the residents) there was a clear correlation between geographic and genetic distance in the North Pacific. And the genetic distance between geographically separate resident populations (e.g. Russian and southern residents, both of which have the ENPSR mtDNA haplotype) was the same as between the sympatric resident and transient populations. The implication is that both allopatry and resource allocation can limit gene flow in this species. The mechanism that reduces gene flow between sympatric foraging specialists is unknown, but could result from differences in reproductive and dispersal strategy that are a consequence of the spatial and temporal patterns of habitat use dictated by the two different foraging behaviors. While this may lead to the local differentiation of populations over the short term, it remains plausible that these behavioral strategies are ephemeral, and that changing environments could lead to new patterns of population structure. If on the other hand resource specialists were on a trajectory towards insipient speciation, the magnitude of genetic differentiation suggests a very recent split, and no greater split than between geographically separated populations of pods that share the same behavior.

A coalescent approach was used to estimate the magnitude of directional geneflow between three populations of particular interest (Southeast Alaskan residents, southern residents and Southeast Alaskan transients). The estimated rate of geneflow per generation was a bit higher than the estimates provided by the private allele method (Tables 4&5), but the relative level among different population pairs was about the same. In each case the apparent level of geneflow was not less between populations of different resource specialization. In fact, the highest level was between southern residents and Southeast Alaskan transients. This could reflect ongoing or historical geneflow, but the lack of a clear difference in magnitude for within vs between type comparisons weakens the argument for greater isolation between types. The Bayesian likelihood method (implemented by STRUCTURE) identified possible migrants between pods of both the same and different foraging behavior. All indications suggest a level of geneflow that is relatively low, perhaps on the order of one migrant every 10-30 years (assuming a generation time of about 30 years).

Conclusions

The level of mtDNA variation is remarkably low for this species, even in the context of small effective population size. Data from this and an earlier study are consistent with a bottleneck hypothesis, though there are other possible scenarios for the loss of mtDNA diversity. These include ideas about the cultural hitch-hiking of the mtDNA genome and a pattern of differential growth rate in matrifocal populations in different parts of the species range (see Whitehead 1998 and online comments in response). I feel that the bottleneck hypothesis best explains the available data. This would likely affect the pattern of haplotypes among populations, reflecting the stochastic redistribution of matriline following a post-bottleneck expansion. This, together with the founding of local populations by matrifocal social groups could explain the regional pattern of mtDNA diversity in the North Pacific (where we have the most data). MtDNA indicates strong philopatry for females, but tells us nothing about the dispersal behavior of males. The analysis of 17 nuclear loci reported here suggests that the level of differentiation among populations is not greater than expected for conspecific populations, and follows a pattern of isolation by distance. At the same time, behavioral specialists show a similar level of differentiation in sympatry. In the context of biased sampling including kin for within population samples, and low effective population size, the magnitude of genetic distance is not large among any of these populations. The most parsimonious interpretation seems to be that there is ongoing or at least very recent male-mediated gene flow among the populations in the North Pacific, including between transients and residents. At the same time, there is clear differentiation both between resource specialists in sympatry and among regional populations. The implication is that all of these populations should be managed as separate management units, and that further research should be undertaken to better define population boundaries.

Acknowledgements

Thanks to my collaborators who provide assistance in the field and in the interpretation of the data, especially Marilyn Dahlheim, Nancy Balck, Ada Natoli, Robin Baird & Carlos

Olavarria. Thanks to my lab technician Colin Nicholson who undertook most of the labwork for this contract. Thanks to NMFS northwest for providing the funding.

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Table Captions:

Table 1: Microsatellite reaction conditions and locus names. All PCR reactions were run for 35 cycles.

Table 2: Expected (H_e) and observed (H_o) heterozygosity estimates per locus and population. SR = southern resident, RU = Russian residents, AR = Southeast Alaskan resident, BS = Bering Sea residents, OS = offshore, AT = Southeast Alaskan transients, CT = Californian transients, ICE = Iceland.

Table 3: Allelic richness and allele number (in parentheses) for each locus and population (population abbreviations are the same as for Table 2).

Table 4: F_{st} values for pairwise population comparisons (lower diagonal). Significance is indicated by asterisks (**** = $p < 0.001$, *** = $p < 0.005$, ** = $p < 0.01$, * = $p < 0.05$). Estimates of gene flow based on the private allele method are given in the upper diagonal.

Table 5: Estimated of gene flow based on the coalescent method implemented in MIGRATE.

Figure Captions:

Figure 1: Location of sample collection sites.

Figure 2: Alignment of haplotypes showing variable sites. Asterisks mark the central conserved domain. Boxes indicate lineages identified in the phylogenetic analyses.

Figure 3: Unrouted neighbour joining tree.

Figure 4: Linearized neighbour joining tree. Kimura two-parameter genetic distances are shown. Bootstrap values based on 1,000 replications.

Figure 5: Mantel test for correlation between geographic and genetic distance among the four putative resident-type populations sampled in the North Pacific.

Figure 6: Proportional assignment to one of seven putative populations (in seven different colors) for each of the 211 individual whales in the study. Population of origin is indicated below the histogram, and putative migrants are indicated above with arrows.

Table 1:

Microsatellite DNA Loci

Locus	Reaction Conditions			Reference
	Standard Taq		Qiagen kit *	
	Anneal (°C)	Mg (mM)	Anneal (°C)	
MK5	46	1.5	60	Krützen et al. (2001)
FCB12	54	1.5	56	Buchanon et al. (1996)
EV1	61-61-57-53	1.2	56	Valsecchi & Amos (1996)
EV5	-	-	62	Valsecchi & Amos (1996)
EV37	50	1.4	56	Valsecchi & Amos (1996)
GATA053	60	0.8	55	Palsboll et al. (1997)
GT48	-	-	50	Palsboll et al. (1997)
GT142	-	-	56	Palsboll et al. (1997)
MK9	-	-	60	Krützen et al. (2001)
GATA098	-	-	56	Palsboll et al. (1997)
FCB11	61-61-57-55	1.5	55 †	Buchanon et al. (1996)
FCB4	58	1.5	62	Buchanon et al. (1996)
KWM12A	42	0.75	62	Hoelzel et al. (1998)
KWM2A	42	0.75	48	Hoelzel et al. (1998)
FCB5	61-61-57-54	1.1	62	Buchanon et al. (1996)
FCB17	61-61-57-55	1.2	56 †	Buchanon et al. (1996)
BA417	42	2.2	53	Schlotterer et al. (1991)

* Qiagen multiplex kit - all at 3mM Mg

† Qiagen kit works best

Table 2:

	SR		RU		AR		BS		OS		AT		CT		ICE	
Locus	Ho	He	Ho	He	Ho	He	Ho	He	Ho	He	Ho	He	Ho	He	Ho	He
MK5	0.786	0.723	0.571	0.722	0.6	0.656	0.789	0.704	0.5	0.605	0.743	0.752	0.762	0.759	0.632	0.626
FCB12	0.476	0.604	0.571	0.611	0.464	0.561	0.45	0.432	0.429	0.593	0.4	0.539	0.647	0.64	0.031*	0.151
EV1	0.368	0.575	0.769	0.68	0.188	0.234	0.357	0.415	0.857	0.758	0.733	0.738	0.684	0.663	0.5	0.546
EV5	0.77	0.613	0.429	0.521	0.297	0.394	0.385	0.462	0.111	0.634	0.719	0.665	0.571	0.681	0.703	0.766
EV37	0.391	0.616	0.385	0.455	0.667	0.741	0.4	0.572	0.333	0.864	0.458	0.654	0.55	0.722	0.606	0.637
FCB4	0.423	0.558	0.643	0.648	0.821	0.812	0.65	0.869	0.6	0.863	0.828	0.862	0.947	0.945	0.667	0.739
KW12a	0.931	0.762	0.786	0.603	0.615	0.661	0.75	0.6	0.2	0.721	0.765	0.776	0.857	0.77	0.825	0.703
KW2a	0.571	0.725	0.462	0.646	0.684	0.532	0.235	0.348	0.889	0.779	0.781	0.805	0.809	0.839	0.75	0.605
FCB5	0.654	0.707	0.286	0.437	0.629	0.699	0.429	0.638	0.714	0.725	0.833	0.754	0.733	0.777	0.622	0.749
FCB17	0.478	0.39	0.231	0.517	0.714	0.608	0.462	0.563	0.667	0.485	0.719	0.517	0.526	0.605	0.464	0.563
BA417	0.28	0.3339	0.385	0.403	0.139	0.228	0.375	0.517	0.375	0.325	0.438	0.571	0.429	0.361	0	0
GATA053	0	0	0	0	0	0	0	0	0	0.314	0.371	0.406	0.25	0.279	0.487	0.486
GT48	0.278*	0.554	0	0	0.688	0.686	0.818	0.662	0.429	0.681	0.677*	0.849	0.421	0.669	0.531*	0.793
GT142	0.655	0.574	0.429	0.545	0.45	0.505	0.737	0.585	0.1	0.368	0.5	0.466	0.818	0.684	0.872	0.638
MK9	0.611	0.654	0	0	0.5	0.646	0.375	0.8	0.75	0.75	0.667	0.723	1	0.762	0.806	0.651
GATA098	0.167	0.197	0.182	0.502	0.077*	0.323	0.118	0.169	0.2	0.284	0.588	0.535	0.35	0.585	0.308	0.339
FCB11	0.786	0.765	0.455	0.697	0.615	0.713	0.833	0.697	0.714	0.747	0.857	0.67	1	0.8	0.929	0.778
Ave Ho	0.507		0.387		0.479		0.480		0.463		0.652		0.668		0.573	
	± 0.247		± 0.247		± 0.249		± 0.248		± 0.279		± 0.161		± 0.227		± 0.264	

Table 3:

<i>Locus</i>	SR	RU	AR	BS	OS	AT	CT	ICE	All
MK5	4.165 (6)	3.407 (4)	3.065 (4)	3.985 (5)	2.936 (3)	4.799 (8)	4.019 (5)	3.443 (5)	4.492 (9)
FCB12	3.314 (4)	2.682 (3)	2.916 (4)	2.49 (3)	3.703 (4)	3.316 (4)	3.304 (4)	1.717 (4)	3.751 (6)
EV1	2.847 (4)	3.439 (4)	2.154 (4)	2.638 (3)	3.978 (4)	4.307 (5)	3.697 (5)	2.644 (4)	3.905 (5)
EV5	3.507 (7)	2.424 (3)	2.818 (6)	3.042 (4)	3.544 (4)	3.627 (6)	3.476 (4)	5.055 (7)	3.975 (10)
EV37	3.655 (5)	2.720 (3)	4.020 (5)	3.312 (4)	4.000 (4)	4.587 (8)	5.215 (9)	3.691 (6)	5.168 (13)
FCB4	3.985 (7)	2.981 (3)	6.243 (17)	7.114 (13)	6.656 (9)	6.941 (15)	8.998 (18)	4.910 (10)	7.324 (29)
KW12a	4.492 (7)	3.896 (5)	4.432 (8)	3.475 (5)	2.996 (3)	4.826 (7)	4.430 (6)	4.172 (7)	4.641 (9)
KW2a	4.213 (6)	3.181 (4)	2.316 (4)	1.945 (2)	4.705 (5)	5.466 (7)	5.613 (7)	2.891 (3)	4.659 (7)
FCB5	3.999 (7)	3.115 (5)	4.169 (7)	3.549 (4)	3.846 (4)	4.693 (9)	4.379 (5)	4.897 (7)	5.016 (16)
FCB17	2.474 (4)	2.456 (3)	3.731 (6)	2.719 (3)	2.000 (2)	2.526 (4)	3.963 (6)	2.627 (3)	3.353 (8)
BA417	1.935 (2)	2.580 (3)	2.045 (3)	2.743 (3)	1.993 (2)	2.859 (3)	2.419 (3)	1.000 (1)	2.418 (4)
GATA053	1.000 (1)	1.000 (1)	1.000 (1)	1.000 (1)	1.902 (2)	2.725 (6)	2.415 (5)	3.012 (5)	2.186 (8)
GT48	(4)	(2)	(3)	(4)	(3)	(4)	(4)	(6)	(8)
GT142	2.980 (3)	1.999 (2)	2.148 (4)	3.107 (2)	2.453 (3)	2.750 (3)	3.251 (3)	3.900 (4)	3.442 (4)
MK9	(4)	NA	(9)	(4)	(4)	(12)	(7)	(10)	(13)
GATA098	1.837 (4)	1.997 (2)	2.509 (5)	1.588 (3)	2.200 (3)	2.176 (7)	2.300 (5)	2.568 (6)	2.693 (7)
FCB11	(7)	(3)	(5)	(4)	(5)	(4)	(4)	(6)	(7)
Ave Allelic Richness	3.095 ± 1.046	2.706 ± 1.314	3.111 ± 1.314	3.050 ± 1.418	3.351 ± 1.306	3.971 ± 1.348	4.106 ± 1.722	3.323 ± 1.208	

Table 4:

	SR	RU	AR	BS	OS	AT	CT	ICE
<i>SR</i>	-	1.69	2.53	2.07	0.45	1.50	1.59	0.98
RU	0.125***	-	2.18	2.06	0.48	0.90	0.79	0.48
AR	0.061****	0.095****	-	3.38	1.06	1.34	0.97	0.69
BS	0.089***	0.052***	0.029**	-	0.53	1.21	0.72	0.62
OS	0.122*	0.201*	0.186**	0.208	-	1.34	0.93	0.68
AT	0.122****	0.132****	0.156****	0.143***	0.106*	-	2.56	0.94
CT	0.131****	0.152****	0.168****	0.166**	0.130*	0.035****	-	1.03
ICE	0.141****	0.245****	0.154****	0.205***	0.148**	0.145****	0.136****	-

Table 5:

From → to	Nm	95% Confidence Limits
SR → AR	1.17	(1.07 – 1.26)
AR → SR	2.47	(2.31 – 2.63)
AR → AT	4.83	(4.58 – 5.14)
AT → AR	3.11	(2.93 – 3.44)
SR → AT	7.88	(7.57 – 8.19)
AT → SR	4.96	(4.74 – 5.18)

Figure 1:

