Crossing to safety: dispersal, colonization and mate choice in evolutionarily distinct populations of Steller sea lions, *Eumetopias jubatus*

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Abstract

Population growth typically involves range expansion and establishment of new breeding sites, while the opposite occurs during declines. Although density dependence is widely invoked in theoretical studies of emigration and colonization in expanding populations, few empirical studies have documented the mechanisms. Still fewer have documented the direction and mechanisms of individual transfer in declining populations. Here, we screen large numbers of pups sampled on their natal rookeries for variation in mtDNA ($n = 1106$) and 16 microsatellite loci ($n = 588$) and show that new Steller sea lion breeding sites did not follow the typical paradigm and were instead colonized by sea lions from both a declining (Endangered) population and an increasing population. Dispersing individuals colonized rookeries in the distributional hiatus between two evolutionarily distinct ($\bar{\Phi}_{st} = 0.222$, $\bar{R}_{st} = 0.053$, $K = 2$) metapopulations recently described as separate subspecies. Hardy–Weinberg, mixed-stock and relatedness analysis revealed levels of interbreeding on the new rookeries that exclude (i) assortative mating among eastern and western forms, and (ii) inbreeding avoidance as primary motivations for dispersal. Positive and negative density dependence is implicated in both cases of individual transfer. Migration distance limits, and conspecific attraction and performance likely influenced the sequence of rookery colonizations. This study demonstrates that resource limitation may trigger an exodus of breeding animals from declining populations, with substantial impacts on distribution and patterns of genetic variation. It also revealed that this event is rare because colonists dispersed across an evolutionary boundary, suggesting that the causative factors behind recent declines are unusual or of larger magnitude than normally occur.

Keywords: colonization, density dependence, microsatellite, mixed-stock, Steller sea lion

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Introduction

The decisions whether to disperse or not and where to settle can have a major influence on individual fitness with consequences for the structure, dynamics and evolution of populations (Clobert *et al.* 2004; Bowler & Benton 2005). As such, patterns of dispersal and settlement have practical implications for the identification of management units, assessment of population viability, demography, and modelling of population response (Clobert *et al.* 2009). The colonization of new habitat patches may involve a suite of motivations, mechanisms and consequences that differ from that involving the

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dispersal of individuals among established groups or populations. Understanding colonization in these terms is especially relevant to investigations on the dynamics and genetic structure of metapopulations (Hanski 1999) and in studies of population decline, recovery and redistribution. Population growth typically involves range expansion and establishment of new breeding sites, while declines are often characterized by range contraction and abandonment of breeding sites (Williams & Giddings 1984; Bradshaw *et al.* 2000; Rodriguez 2002). Although density dependence is widely invoked in theoretical studies and has been implicated in empirical studies of emigration and colonization in expanding populations (Bradshaw *et al.* 2000; Gaggotti *et al.* 2002; Altwegg *et al.* 2013), few empirical studies have documented the mechanisms of this phenomenon. Still fewer have documented the direction and mechanisms of individual transfer in declining populations, or the consequences of colonization in terms of survival, breeding success and genetic diversity.

Here, we report on a genetic study of dispersal, colonization and mate choice in a marine mammal, the Steller sea lion, *Eumetopias jubatus*, involving two neighbouring populations with very different population trends and histories. We use genetic profiling of newborn pups from established and new breeding sites to explore the causes, mechanisms and consequences of dispersal and colonization. Steller sea lions aggregate on remote terrestrial sites, called rookeries, across the north Pacific rim from California to Japan to breed and nurse their young (Loughlin 2002). Characterized by strong sexual dimorphism and a polygynous mating system, large dominant males establish and defend breeding territories each summer while the much smaller females give birth, nurse their pups and breed with territorial bulls. Population trends over the past four decades have differed widely among regions. Dramatic declines, followed by moderate recovery in some areas, have occurred throughout the Gulf of Alaska and Aleutian Islands compared to steady increases in southeast Alaska and British Columbia (Merrick *et al.* 1987; Trites & Larkin 1996; Pitcher *et al.* 2007; Matthews *et al.* 2011; Fritz *et al.* 2013; Fig. 1). The causes of the decline appear to be multifactorial (National Research Council
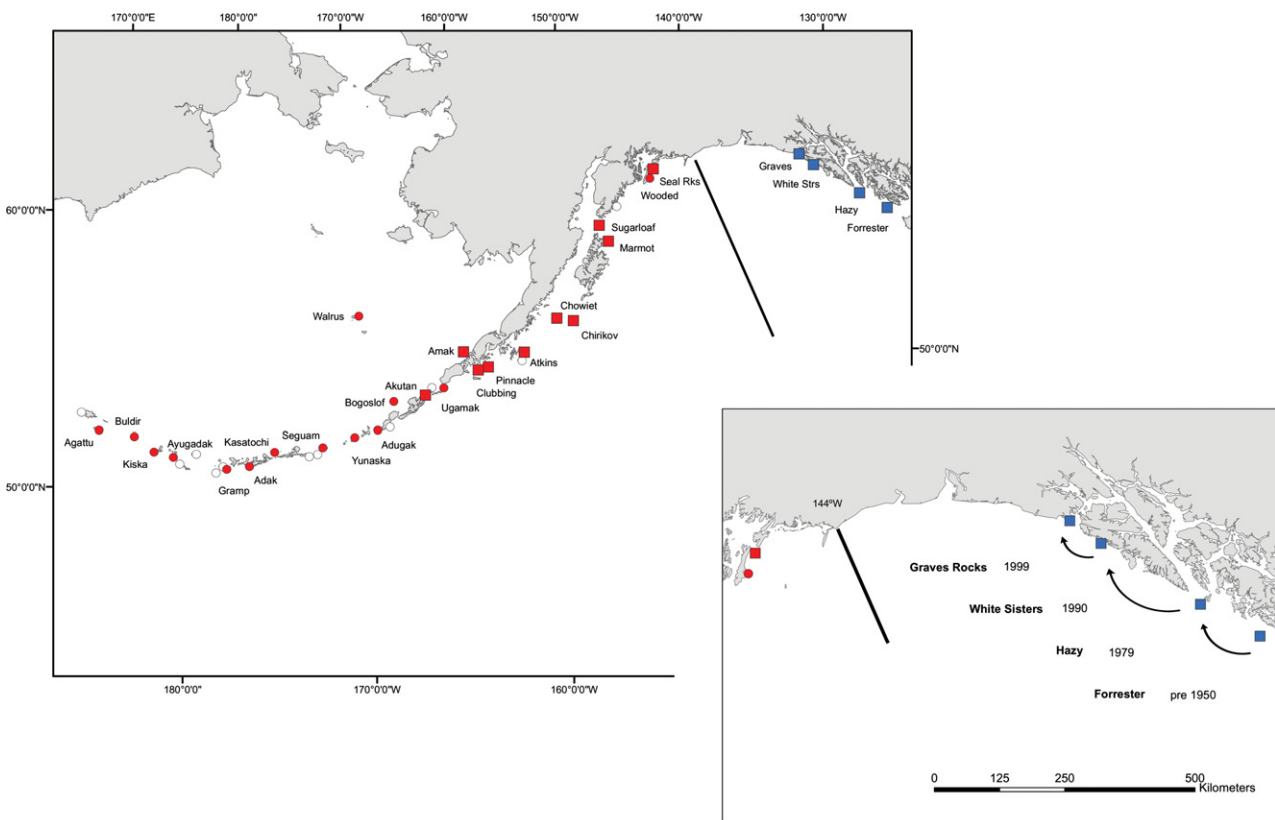

Fig. 1 Map of Steller sea lion rookeries in Alaska, including those (in colour; western in red, eastern in blue) investigated in this study. The black line denotes the boundary between the eastern and western Distinct Population Segments at 144°W. Square symbols represent rookeries where pups were analysed for both mtDNA and microsatellites. Inset details the dates and hypothesized origins (arrows) of colonization of new rookeries in the eastern DPS.

2003; Atkinson *et al.* 2008), and investigations of these diverging trends and the subsequent designation of management units have been fuelled by legal mandates for species recovery and the potential for enormous economic impact of management decisions on the fishing industry in Alaska (NRC 2003; Dalton 2005).

A phylogeographic study of mtDNA variation revealed two evolutionarily distinct populations of Steller sea lions (Bickham *et al.* 1996) coincident with the observed broad-scale differences in population trajectory, prompting the U.S. National Marine Fisheries Service to define a western and eastern Distinct Population Segment (DPS) or stock under the Endangered Species Act with a boundary in the distributional break at 144°W (Loughlin 1997; Fig. 1) and to list them as 'endangered' and 'threatened', respectively (U.S. Fed Reg. 1997; Loughlin 1997). Variable trends and moderate natal fidelity observed on subregional scales, compared to clear genetic and population trajectory differences, a 900 km hiatus in breeding distribution, and low recorded trans-boundary movement at the DPS level indicate that the eastern and western populations are best viewed as two independent metapopulations each comprised of a series of local populations connected by dispersal (York *et al.* 1996; Raum-Suryan *et al.* 2002).

Additional mtDNA studies confirmed the primary evolutionary break in the eastern Gulf of Alaska, dated the mtDNA split to the Pleistocene, and documented structure at smaller spatial scales (Bickham *et al.* 1998; Trujillo *et al.* 2004; Baker *et al.* 2005; Harlin-Cognato *et al.* 2006; O'Corry-Crowe *et al.* 2006; Phillips *et al.* 2011). A series of microsatellite studies found varying levels of subdivision, the most extensive documenting two population clusters that were geographically concordant with the eastern and western stocks based on mtDNA studies (Trujillo *et al.* 2004; Hoffman *et al.* 2006). This was taken as support for an ancient population break with little subsequent male or female dispersal over time (Hoffman *et al.* 2006). The recent documentation of craniometrical differences coincident with the primary genetic break has led to the description of an east and west form of Steller sea lion and a proposal that they can be elevated to subspecific rank: *E.j. monteriensis* and *E. j. jubatus*, respectively (Phillips *et al.* 2009).

Despite this emerging picture of long-established population subdivision at or near the 144°W meridian, population trends and founder events indicate that the dispersal patterns and breeding behaviour of Steller sea lions continue to evolve, especially in the boundary region. While most eastern and western animals were assigned (i.e. $q > 0.5$) to their respective genetic clusters in Hoffman *et al.*'s (2006) microsatellite study, over one-third (36.1%) of the western individuals were assigned to the eastern cluster. Additionally, the nDNA studies had low sample sizes ($n \leq 10$) for most rookeries near the population boundary (i.e. eastern Gulf of Alaska to northern southeast Alaska) and all prior genetic investigations included almost no samples from the newest rookeries (see below). Similarly, some overlap was observed in skull morphology among populations by Phillips *et al.* (2009) and that study contained very few specimens from more than 3000 km of coastline between the central Gulf of Alaska and California, including almost the entire extant range of the eastern population.

The population history of the boundary region has also been quite dynamic. In recent decades, population trends on rookeries to the immediate west of 144°W have been somewhat asynchronous with the declining trends or slow recovery on most other western breeding sites, with pup production on rookeries such as Seal Rocks (Fig. 1) growing from very small numbers in the 1960s to several hundred by the early 1990s (Trites & Larkin 1996), a trend that continues today (Fritz *et al.* 2013). East of 144°W, exponential growth in southeast Alaska has been accompanied by the sequential colonization of new breeding sites north of Forrester Island, the only rookery in southeast Alaska for most of the 20th century (Rowley 1929; Trites & Larkin 1996; Pitcher *et al.* 2007). These new rookeries are as follows: Hazy Island (1979), White Sisters (1990) and Graves Rocks (1999), and Biali Rocks (early 2000s: Pitcher *et al.* 2007; Matthews *et al.* 2011; Fig. 1).

The colonization of new breeding habitat in the distributional hiatus between two populations with opposing trends has implications for sea lion management including population assessment and the delineation of stocks. It also provides a unique opportunity to investigate the causes, mechanisms and consequences of founding events in a large mammal. Both the timing and sequence of colonizations in southeast Alaska fit the classic positive density-dependent model of population expansion with limited individual migration distances. Population decline in the rest of Alaska has been characterized by a reduction in pup production and overall numbers at rookeries, and some rookery extinctions (Fritz *et al.* 2013). Endogenous population regulation models would predict limited dispersal from western DPS rookeries. The location of the new rookeries, however, also provides new breeding opportunities for colonists where inbreeding avoidance may promote emigration from both populations. Range expansion into the distributional hiatus between two evolutionarily distinct populations that have also been characterized as distinct morphotypes and presumptive subspecies could further resolve the basis of such

genetic, morphological and taxonomic distinctions by investigating whether differences are maintained via assortative mating among phenotypes (Roughgarden 1979; Schluter 2009) on the new breeding sites.

We examined patterns of variation within 531 bp of the mtDNA control region in over 1100 Steller sea lion pups and within 16 microsatellite markers in 588 pups from 11 long-established rookeries and three newer rookeries in the Gulf of Alaska (western DPS) and southeast Alaska (eastern DPS). We also used published results from an mtDNA study of 592 individuals, including 443 from 12 rookeries across the rest of the species Alaska range (O'Corry-Crowe *et al.* 2006), in some analyses. Considering earlier findings on dispersal over evolutionary time frames and the extensive distances between long-established rookeries in the eastern and western DPS, and assuming positive density-dependent dispersal and limited dispersal distance in this species, we predicted: (i) low levels of contemporary gene flow across the DPS boundary, (ii) if some gene flow does occur, it will primarily take place among rookeries in the boundary region and (iii) that new rookeries to the east of 144°W were colonized sequentially by animals from the growing eastern DPS. If the motivation for colonization is primarily inbreeding avoidance, we predicted: (iv) colonizing sea lions would preferentially mate with individuals originating from the other source population. If observed phenotypic differences among source populations represent distinct geographic morphotypes, positive assortative mating predicts: (v) that colonizing sea lions on new rookeries will breed primarily with individuals from their natal population regardless of the location.

Materials and methods

Skin plug samples were collected from newborn pups ($n = 1106$; $\bar{n}_{rookery} = 79$) from long-established rookeries in both the western and eastern DPS and from three new rookeries in the distributional hiatus in the western Gulf of Alaska and southeast Alaska (Table 1, Fig. 1). All pups were sampled within 3 weeks of birth, ensuring that sampling occurred at the rookery of birth. Tissues were preserved in 20% DMSO saturated in NaCl or in 90% Ethanol. Methods used to extract total DNA and to amplify and sequence 531 bp of the mtDNA control region were reported earlier (O'Corry-Crowe *et al.* 2006).

We optimized PCR conditions for 36 independent microsatellite loci typed on a number of phocid and otariid species (Hoelzel *et al.* 1999; Allen *et al.* 1995; Coltman *et al.* 1996; Goodman 1997; Gemmell *et al.* 1997; Davis *et al.* 2002; Hernandez-Velazquez *et al.* 2005), including several previously used on *E. jubatus* (Trujillo *et al.* 2004; Hoffman *et al.* 2006), and tested on a subset of individuals. We investigated the incidence of null alleles and scoring errors, including allelic dropout, via repeat screening of individual genotypes, and

Table 1 Sample size of Steller sea lion pups sampled on rookeries in the eastern and western DPS and screened for variation in mtDNA and 16 microsatellite loci. The study focussed on pups from Southeast Alaska west to the eastern Aleutian Islands and Bering Sea (column 3 and 4). Samples used in the construction of a median joining network of mtDNA lineages across the species Alaskan range are given in column 5

Rookery	DPS	Microsatellites	mtDNA	mtDNA -MJN	Geo. coordinates
Forrester	Eastern	69	141	141	54° 51.7′ N 133° 32.2′ W
Hazy	Eastern	82	129	129	55° 52.0′ N 134° 34.0′ W
White Sisters	Eastern	95	180	180	57° 38.1′ N 136° 15.4′ W
Graves Rocks	Eastern	94	93	93	58° 14.3′ N 136° 45.4′ W
Seal Rocks	Western	36	80	80	60° 9.78′ N 146° 50.3′ W
Fish/Wooded	Western			56	59° 52.9′ N 147° 20.7′ W
Sugarloaf	Western	48	64	64	58° 53.2′ N 152° 2.4′ W
Marmot	Western	20	67	67	58° 13.7′ N 151° 47.8′ W
Chirikov	Western	9	65	65	55° 46.5′ N 155° 39.5′ W
Chowiet	Western	24	24	24	56° 0.56′ N 156° 41.4′ W
Atkins	Western	32	56	56	55° 3.20′ N 139° 17.4′ W
Pinnacle	Western	31	51	51	54° 46.1′ N 161° 45.9′ W
Clubbing Rocks	Western	24	25	25	54° 52.8′ N 162° 26.7′ W
Ugamak	Western			93	54° 13.5′ N 164° 47.5′ W
Akutan	Western	5	78	78	54° 3.39′ N 165° 59.7′ W
Amak	Western	19	53	53	55° 27.8′ N 163° 12.1′ W
Walrus - Agattu	Western			443	
	Total	588	1106	1698	

the testing of genotypic data for deviations from Hardy–Weinberg (H-W) expectations via MCMC bootstrapping (10 000 reps) in MICROCHECKER (v. 2.3.3; Van Oosterhout *et al.* 2004) and MCMC exact tests (100 000 iterations) in GENEPOP (v. 4.1; Rousset 2008). Linkage disequilibrium among microsatellite loci was tested via permutation (20 000 runs) in ARLEQUIN (v. 3.5, Excoffier & Lischer 2011) and MCMC exact tests (500 000 iterations) in GENEPOP. Ultimately, a total of 16 loci were chosen for multilocus genotyping ($n = 588$ pups; $\bar{n}_{rookery} = 42$; Table 2). Diversity indices, including the number of alleles and haplotypes, heterozygosity (H) and probabilities of identity (P_{ID}) for each rookery were calculated using ARLEQUIN and the program Doh (Brzustowski 2002).

We constructed a median-joining network (MJN) of mtDNA haplotypes using Network (v. 4.6.1; Fluxus Technology Ltd. 2011). To achieve the shortest and least complex network of most parsimonious trees, we used a range of character weighting schemes, including downweighting characters with high mutation rates (e.g. from 10 down to 5 or 0) and testing different transition to transversion weight ratios (ts:tv = 1:1–3:1), and tried a range of values (0–10) for the epsilon parameter.

Genetic differentiation was assessed via Wright's (1965) frequency-based parameter F_{st} and its distance-based analogues Φ_{st} and R_{st} for mtDNA and microsatellites, respectively, using an analysis of variance approach (Weir & Cockerham 1984; Excoffier *et al.* 1992) in ARLEQUIN. Permutation-based homogeneity tests (50 000 runs) were performed, and confidence intervals

of parameter estimates for microsatellite data were estimated by bootstrap analysis (20 000 reps) across loci. Population subdivision was also investigated via the model-based clustering algorithm, STRUCTURE, (v.2.3.4) which uses a Bayesian approach to estimate the most likely number of population clusters, K, given the data (Pritchard *et al.* 2000). Both admixture and no-admixture models were applied. MCMC methods were used to integrate over the parameter space, and multiple long runs ($n = 10$ of 50 000 burn-in followed by 1×10^6 reps) with different starting conditions were conducted and summary statistics monitored for convergence. Prior information on sample group was used to help resolve population structure (LOCPRIOR model; Hubisz *et al.* 2009). This new approach, not available in earlier nDNA studies of Steller sea lions, has been found to be better able to detect underlying structure without risking detection of subdivision that is not present (Hubisz *et al.* 2009). Contemporary dispersal and genetic exchange were also examined via identification of migrants (in this case, pups where both parents were migrants from the same population) and individuals with shared ancestry using assignment methods and by estimating rates of recent migration. Assignment probabilities to and admixture proportions (Q) from each of K populations were estimated for each individual in STRUCTURE. Traditional likelihood methods (Paetkau *et al.* 1995) were also used in the program WHICHRUN (v. 4.1; Banks & Eichert 2000), which estimates the likelihood of an individual's haplotype or multilocus genotype coming from each of B populations defined a priori

Table 2 Details on the microsatellite loci screened in the study of Steller sea lion dispersal and colonization, including original refrences, allelic size range, basic diversity indices and estimated probabilities of identity

Name	Reference	Number of alleles	Allele size range (base pairs)	H_O	H_E	P_{ID}
Aa4	Gemmell *et al.* (1997)	3	214−220	0.446	0.495	0.350
Hg3.7	Allen *et al.* (1995)	6	402−412	0.619	0.620	0.180
Hg4.2	Allen *et al.* (1995)	10	160−186	0.542	0.570	0.236
Hg6.3	Allen *et al.* (1995)	6	228−238	0.193	0.205	0.638
Hl4	Davis *et al.* (2002)	9	141−157	0.687	0.684	0.152
Lc28	Davis *et al.* (2002)	9	135−165	0.657	0.664	0.162
M2b	Hoelzel *et al.* (1999)	12	91−119	0.842	0.839	0.045
M11a	A. R. Hoelzel, unpublished	9	148−170	0.816	0.827	0.052
Pv16	Goodman (1997)	5	130−138	0.712	0.682	0.164
Pvc29	Coltman *et al.* (1996)	8	127−143	0.735	0.742	0.102
Pvc78	Coltman *et al.* (1996)	8	158−172	0.324	0.333	0.465
Zc1.8	Hernandez-Velazquez *et al.* (2005)	6	170−180	0.619	0.639	0.183
Zc4.7	Hernandez-Velazquez *et al.* (2005)	5	248−258	0.676	0.673	0.175
Zc48	Hernandez-Velazquez *et al.* (2005)	6	255−265	0.486	0.514	0.309
Zc5.8	Hernandez-Velazquez *et al.* (2005)	11	317−347	0.807	0.806	0.062
Zc7tg	Hernandez-Velazquez *et al.* (2005)	8	278−294	0.604	0.619	0.206
Overall		7.56		0.610	0.620	5.373×10^{-13}

based on observed haplotype and allele frequencies. We investigated the utility of the program BAYESASS (v. 1.3; Wilson & Rannala 2003) to accommodate source-colony dynamics when estimating the magnitude and direction of recent migration.

Mate choice on new rookeries was assessed by comparing estimates of average relatedness (based on nDNA) within and between groups of pups of eastern and western maternal ancestry (i.e. based on mtDNA). Using the program COANCESTRY (Wang 2011), seven different moment and likelihood estimators of relatedness, r, were calculated from the multilocus genotype data, including those of Queller & Goodnight (1989) and Wang (2002). Bootstrap analysis (100 000 reps) over loci was used to obtain confidence in intrapair estimators and intergroup comparisons of average relatedness.

Finally, population mixing and genetic introgression on new rookeries were investigated via mixed-stock analysis using the program BAYES (Masuda 2002). This Bayesian method makes use of the stock-mixture samples and similarities among baseline stocks to improve estimation of Q, the relative frequencies of haplotypes, alleles and genotypes in the baselines, and thus improves determination of the origins of mixture samples (Pella & Masuda 2001). Convergence on the posterior distributions of stock-mixture proportions, p, and individual assignments was achieved by running multiple independent MCMC chains ($\geq$1500 steps/chain) with different starting stock-mixture proportions and low (<1.2) shrink factors.

Results

We found 62 variable sites defining 130 unique haplotypes within mtDNA in 1698 individuals from across the Alaskan range of Steller sea lions from Forrester Is. (southeast Alaska) to Agattu Is. (western Aleutian Islands). The MJN was characterized by a series of interconnected star-like phylogenies with common haplotypes occupying more central positions within the network and rarer haplotypes at the branch tips (Fig. 2). Allelic diversity, H_O, H_E and P_{ID} varied across microsatellite loci (Table 2). Only four of 224 tests across 16 loci and 14 rookeries revealed an excess of homozygotes in MICROCHECKER while nine of 224 tests were adjudged to deviate significantly ($P < 0.05$) from H-W expectations using GENEPOP. The low number of significant tests had no consistent pattern across loci or rookery indicating no inherent problems with null alleles nor scoring errors in the loci screened. All loci were found to be in linkage equilibrium. MtDNA and microsatellite diversity estimates varied across the 14 rookeries within the eastern DPS and the shelf region of the western DPS that are the primary focus of the current study (Table 3).

mtDNA differentiation between eastern and western DPS

Table 4 summarizes patterns of differentiation among the 14 eastern and western-shelf rookeries that were investigated for mtDNA and microsatellite variation.
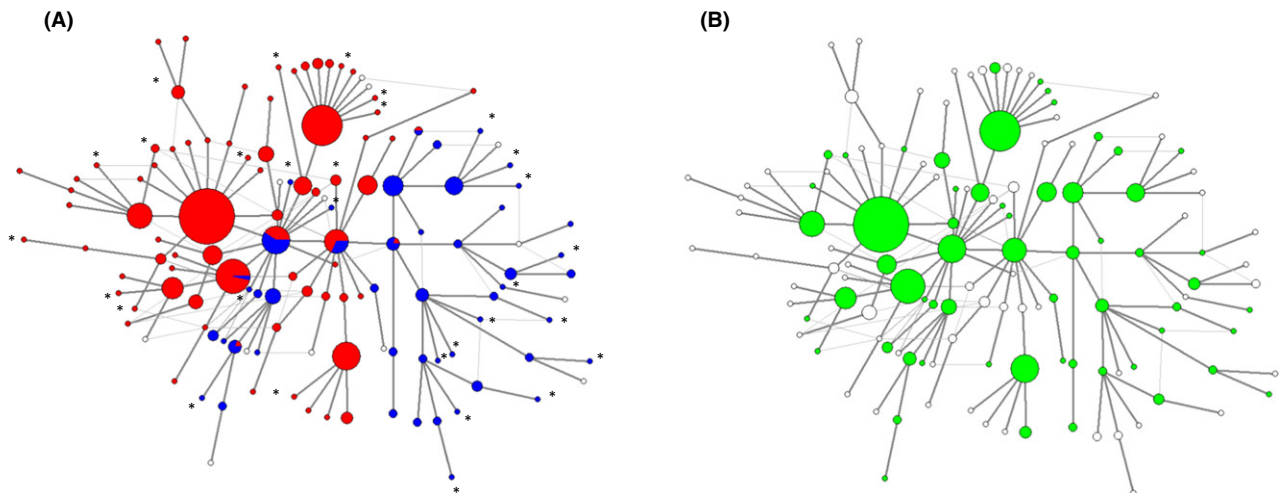

Fig. 2 A median-joining network of 130 Steller sea lion mtDNA haplotypes (character weight = 10, hypervariable characters downweighted to 5, ts:tv = 3:1, epsilon parameter = 10). (A) Haplotypes found in the western and eastern stocks are highlighted in red and blue, respectively. (B) Haplotypes found in the two newest rookeries in southeast Alaska, White Sisters and Graves Rocks, are highlighted in green. Note, haplotypes marked with an asterisk in A were recorded only once in baseline sample sets of equal size ($n = 270$) in likelihood assignment tests (see text for details).

Table 3 Estimates of genetic diverity in mtDNA and microsatellites in Steller sea lion pups from 14 rookeries in the eastern and western DPS, including three rookeries (italicized) established since 1979

Rookery	DPS	Mitochondrial DNA			Microsatellites				
		n	Haplotypic diversity	Nucleotide diversity	n	H_O	H_E	Mean no. alleles/locus	P_{ID}
Forrester	Eastern	141	0.936	0.0063	69	0.588	0.591	5.56	3.2179×10^{-12}
Hazy	Eastern	129	0.944	0.0063	82	0.588	0.583	5.94	2.1642×10^{-11}
White Sisters	Eastern	180	0.962	0.0065	95	0.620	0.603	5.81	1.3713×10^{-12}
Graves Rocks	Eastern	93	0.943	0.0066	94	0.622	0.620	5.94	4.4575×10^{-13}
Seal Rocks	Western	80	0.857	0.0055	36	0.615	0.611	5.38	4.3602×10^{-13}
Sugarloaf	Western	64	0.876	0.0049	48	0.622	0.611	5.69	4.1189×10^{-13}
Marmot	Western	67	0.843	0.0049	20	0.630	0.628	4.69	8.7441×10^{-14}
Chirikov	Western	65	0.873	0.0049	9	0.653	0.591	4.00	1.8941×10^{-13}
Chowiet	Western	24	0.826	0.0047	24	0.569	0.577	4.69	2.3094×10^{-12}
Atkins	Western	56	0.898	0.0050	32	0.612	0.631	5.50	1.4437×10^{-13}
Pinnacle	Western	51	0.895	0.0055	31	0.622	0.623	4.94	1.3477×10^{-13}
Clubbing	Western	25	0.753	0.0043	24	0.619	0.627	4.88	1.0715×10^{-13}
Akutan	Western	78	0.906	0.0058	5	—	—	—	—
Amak	Western	53	0.840	0.0048	19	0.608	0.598	4.75	1.1025×10^{-12}

Ignoring the two newest rookeries (White Sisters and Graves Rocks) for the moment, earlier analyses revealed low mtDNA differentiation between the long-established Forrester Island rookery and Hazy Island, the first of the new rookeries in southeast Alaska ($F_{st} = 0.004$, $\Phi_{st} = 0.00$) and the closest to Forrester (Fig. 1). Negligible mtDNA subdivision was also observed among long-established rookeries within the western DPS ($F_{st} \leq 0.03$, $\Phi_{st} \leq 0.02$). By contrast, substantial mtDNA subdivision ($F_{st} = 0.06$–0.14, $\Phi_{st} = 0.18$–0.26) was observed between the eastern and western rookeries, and higher estimates for the distance-based compared to frequency-based parameter indicated clear phylogeographic partitioning of lineages at the population level. This strong phylogeographic signal between Forrester and Hazy in the east and rookeries in the west is also evident in the MJN of mtDNA haplotypes described from across the entire Alaskan range (Fig. 2A) where, apart from a few central haplotypes common to both populations, mtDNA lineages are almost exclusively found in either the eastern or western population.

Assignment tests between the eastern rookeries of Forrester and Hazy (East, $n = 270$) and a range of western rookery configurations: (west Alaska, $n = 1156$; west shelf, $n = 712$, west shelf – equal sample size, $n = 270$) were conducted in WHICHRUN. In each analysis, few individuals were assigned to the population other than the one where they were sampled (Fig. 3A). For the analysis with equal sample size (i.e. $n = 270$), of 17 cross assignments, the haplotype either had a central position in the network and was common to both popu-

lations ($n = 11$) or was found in rookeries closest to the population boundary (i.e. Hazy Is and Seal Rocks; $n = 6$). Several individuals ($n = 30$) could not be assigned with confidence because they appeared only once, effectively resulting in a tie. Considering the strong phylogeographic signal differentiating east and west (Fig. 2A), we reasoned that a rare haplotype could be assigned to a population of origin if its immediate ancestral haplotype(s) was found exclusively or predominantly in one population. Applying this 'phylogeographic' approach, all 17 individuals with a unique haplotype that were sampled from eastern rookeries (Fig. 2A, highlighted with asterisks) were assigned to the east. Similarly, the unique (or rare) haplotypes only sampled on western rookeries ($n = 13$) were on subclades dominated by other western haplotypes.

Microsatellite differentiation between eastern and western DPS

Heterogeneity was also observed at microsatellite loci at the population and, in some cases, rookery level. Excluding the newest rookeries (White Sisters and Graves Rocks) for now, the frequency-based parameter F_{st} indicated limited gene flow among established rookeries in the eastern and western population ($\bar{F}_{st} = 0.032$, Table 4B). Larger mean pairwise R_{st} compared to F_{st} values among these breeding sites in the two populations ($\bar{R}_{st} = 0.053$) indicates restricted gene flow for periods of time long enough for average allele size to diverge via drift and mutation. In contrast to interpopulation patterns, few inter-rookery differences within

Table 4 Population differentiation within: (A) mitochondrial DNA and (B) across 16 microsatellite loci in Steller sea lions. Values for the frequency-based statistic, F_{st} are below the diagonal, values for the distance-based statistics, Φ_{st} (mtDNA) and R_{st} (nDNA), are above the diagonal. n denotes sample size, and shaded cells represent statistical significance from permutation-based (50 000 runs) homogeneity tests

A. Mitochondrial DNA

	Forrester	Hazy	White Sisters	Graves Rocks	Seal Rocks	Sugarloaf	Marmot	Chirikov	Chowiet	Atkins	Pinnacle	Clubbing Rocks	Akutan	Amak
$n=$	141	129	180	93	80	64	67	65	24	56	51	25	78	53
Forrester		−0.001	0.060	0.122	0.240	0.233	0.263	0.246	0.239	0.198	0.214	0.256	0.210	0.235
Hazy	0.004		0.043	0.099	0.216	0.211	0.240	0.222	0.215	0.176	0.189	0.232	0.188	0.211
White Sisters	0.013	0.006		0.020	0.088	0.081	0.105	0.095	0.087	0.056	0.074	0.100	0.062	0.084
Graves Rocks	0.043	0.036	0.011		0.024	0.031	0.048	0.039	0.023	0.009	0.009	0.050	0.017	0.027
Seal Rocks	0.102	0.096	0.053	0.016		−0.001	0.003	0.011	−0.012	0.002	0.001	0.013	0.000	−0.003
Sugarloaf	0.087	0.080	0.041	0.019	−0.001		−0.007	−0.004	−0.019	−0.002	0.012	−0.006	0.001	−0.005
Marmot	0.109	0.104	0.058	0.028	−0.003	−0.007		−0.004	−0.018	0.011	0.019	−0.017	0.006	−0.005
Chirikov	0.091	0.085	0.044	0.021	0.003	−0.003	−0.005		−0.019	0.005	0.013	−0.012	0.011	−0.003
Chowiet	0.111	0.106	0.064	0.029	−0.006	−0.010	−0.011	−0.004		−0.005	−0.006	−0.016	0.000	−0.019
Atkins	0.074	0.067	0.031	0.008	0.002	−0.005	0.002	−0.001	0.001		−0.007	0.009	−0.004	−0.005
Pinnacle	0.080	0.074	0.037	0.002	−0.002	0.000	0.003	0.002	−0.002	−0.010		0.021	0.003	0.000
Clubbing Rocks	0.142	0.138	0.088	0.059	0.017	0.006	−0.005	−0.004	−0.006	0.012	0.020		0.009	−0.010
Akutan	0.071	0.064	0.026	0.009	0.014	0.013	0.015	0.011	0.030	0.000	0.005	0.031		0.002
Amak	0.103	0.097	0.058	0.034	0.002	−0.001	−0.001	0.000	−0.011	0.007	0.011	0.002	0.023	

B. Microsatellites

	Forrester	Hazy	White Sisters	Graves Rocks	Seal Rocks	Sugarloaf	Marmot	Chirikov	Chowiet	Atkins	Pinnacle	Clubbing Rocks	Akutan	Amak
$n=$	69	82	95	94	36	48	20	9	24	32	31	24	5	19
Forrester		0.001	0.010	0.016	0.029	0.054	0.065	0.100	0.063	0.031	0.061	0.067	0.121	0.072
Hazy	0.000		−0.001	0.008	0.011	0.028	0.039	0.075	0.034	0.013	0.037	0.043	0.076	0.047
White Sisters	0.003	0.000		0.001	0.000	0.017	0.019	0.056	0.015	0.003	0.020	0.025	0.046	0.032
Graves Rocks	0.014	0.012	0.007		0.006	0.014	0.020	0.034	0.014	−0.001	0.012	0.018	0.055	0.018
Seal Rocks	0.021	0.015	0.007	0.002		−0.001	−0.005	0.035	0.001	−0.004	0.008	0.003	0.016	0.021
Sugarloaf	0.027	0.021	0.012	0.002	−0.001		−0.001	0.012	0.009	−0.003	0.007	0.007	0.020	0.022
Marmot	0.039	0.033	0.023	0.017	−0.002	0.007		0.020	−0.005	0.001	−0.002	−0.012	−0.021	0.011
Chirikov	0.034	0.03	0.024	0.004	0.003	−0.002	0.014		−0.005	0.012	0.027	0.002	0.055	0.039
Chowiet	0.035	0.029	0.019	0.006	−0.003	−0.001	0.008	0.009		0.002	−0.005	0.013	−0.014	0.005
Atkins	0.034	0.031	0.018	0.004	−0.001	−0.002	0.008	0.005	0.002		0.000	−0.007	0.022	0.004
Pinnacle	0.035	0.033	0.019	0.007	0.000	−0.001	0.003	0.007	0.001	−0.004		0.002	0.001	−0.009
Clubbing Rocks	0.038	0.035	0.023	0.006	0.000	0.001	0.004	−0.001	0.004	−0.004	−0.003		0.004	0.016
Akutan	0.048	0.037	0.025	0.015	0.006	0.007	−0.01	0.009	0.013	−0.004	0.002	−0.004		0.013
Amak	0.036	0.032	0.02	0.01	−0.002	0.011	0.012	0.025	0.009	0.006	0.006	0.001	0.013	

0.05 ≥ P > 0.01
P ≤ 0.01

(A) mtDNA
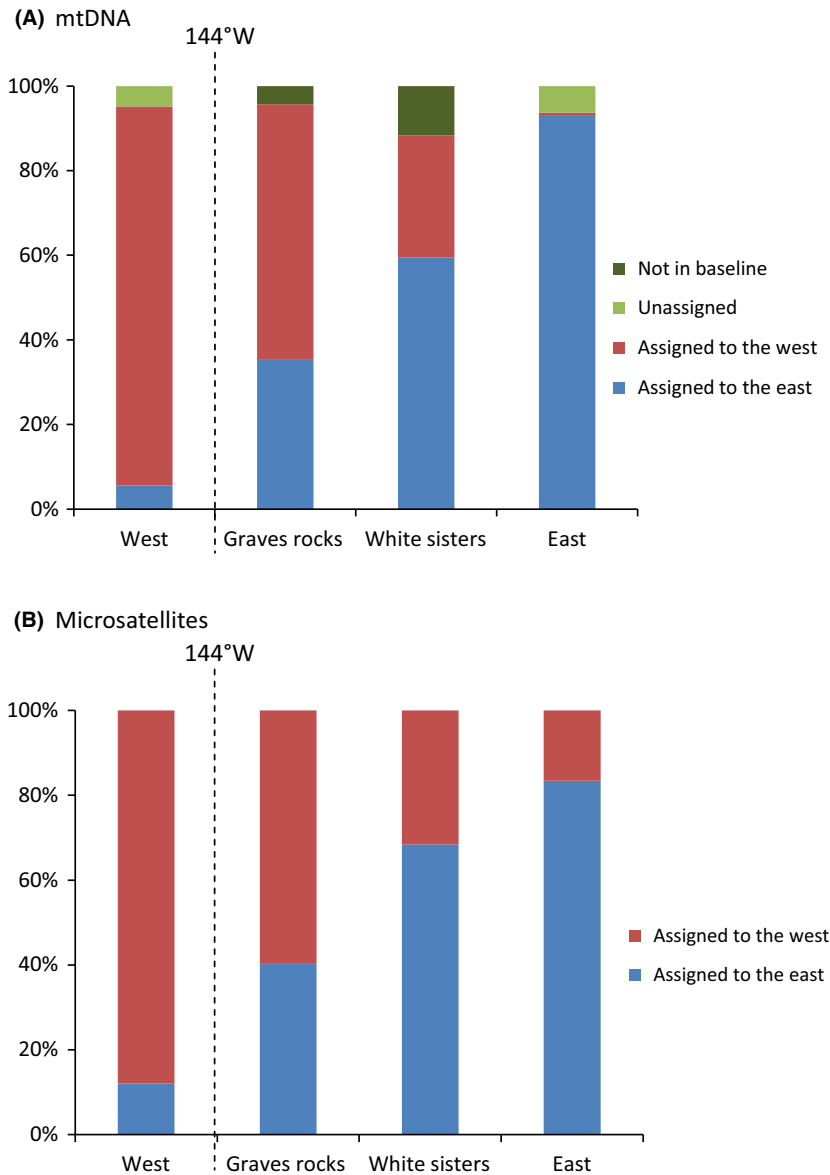

(B) Microsatellites

Fig. 3 Likelihood-based assignment tests of Steller sea lion pups on established and newest rookeries in the western and eastern metapopulations. Established rookeries in both the eastern (Forrester and Hazy) and western (Seal Rocks to Akutan) metapopulations were used as baseline populations. (A) Assignments based on mtDNA haplotype, using equal sample sizes in baseline populations. Those individuals in the baseline population that possessed a unique haplotype are highlighted in light green. Those individuals in the newest rookeries that possessed haplotypes not present in the baseline sample sets are in dark green. (B) microsatellites.

populations were detected. Amak and Chirikov were the exceptions, the latter likely due, at least in part, to its small sample size ($n = 9$).

The STRUCTURE analysis found $K = 2$ population clusters the most likely given the data (Pr $(K|X) \approx 1.0$, Fig. 4). These two clusters are geographically concordant with established breeding sites in the eastern and western DPSs. Incorporating sample group information as a prior improved the discrimination substantially with most individuals having high ancestry proportions (admixture model) or assignment probabilities (no-admixture model) on the order of $q > 0.8$, for a single population cluster (Fig. 5A). Allowing for admixture, the analysis identified a small number of individuals of likely mixed origin. Most of these were sampled at

rookeries in the boundary region: eight pups from Hazy Island in the eastern DPS had substantial admixture proportions ($q_k = 0.33–0.51$) from the west. Similarly, three pups from Seal Rocks and one from Sugarloaf in the western DPS had high estimated admixture proportions ($q_k = 0.32–0.47$, Fig. 5A) from the east. Under a model without admixture, a number of these same individuals had high estimated assignment probabilities ($P_k = 0.34–0.84$) to the population cluster other than where they were sampled.

This pattern of strong discrimination between eastern and western DPS rookeries with a number of cross assignments on rookeries near the boundary was also evident in the WHICHRUN analysis with 13.8% (55/399) of pups with higher estimated likelihoods of arising in the
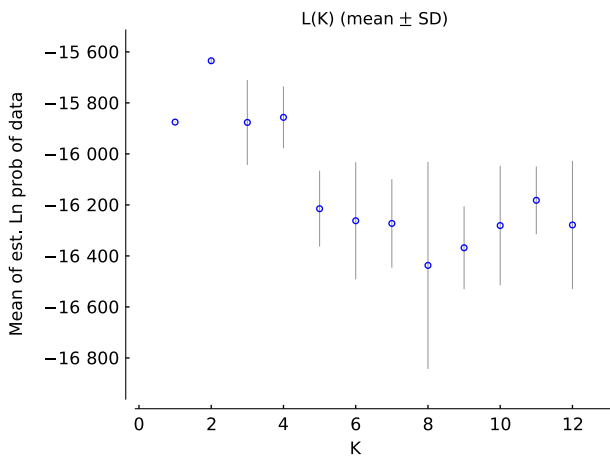
L(K) (mean ± SD)

Fig. 4 Likelihood of the number of population clusters, *K*, given the data using STRUCTURE. Mean (±SD) of the log probability of the data, *Ln P(D)*, over five runs for different values of *K* when using prior sample group location and the admixture model of ancestry. Graph was produced using the Structure Harvester program.

population other than where it was sampled (Fig. 3B). Using a ratio of likelihood functions in excess of 10:1 (or Log ratio, LOD > 1) as the selection criterion for confidence in cross assignments, however, only 9/399 pups (2.25%), four from the east (Hazy, Forrester) and five from the west (Seal Rocks, Sugarloaf and Atkins), were cross-assigned to the other population.

Genetic origins of pups on newest rookeries

The genetic analysis revealed that the two newest rookeries in the eastern DPS, White Sisters and Graves Rock, were not founded solely by eastern animals. Contrary to predictions, pups on the recently colonized rookeries possessed haplotypes characteristic of the western as well as eastern DPS (Fig. 2B). This was also evident in estimates of genetic differentiation for both microsatellite and mtDNA markers (Table 4) where a clear break between rookeries to the west and east of 144°W was expected but not observed, and in assignment tests where several pups on the new rookeries had higher likelihoods of having western than eastern origins (Figs 3 and 5). The BAYES analysis also provided clear evidence of mixing of eastern and western sea lions on the two new rookeries. In most cases, posterior densities of stock proportions spanned intermediate ranges ($\bar{x}$ = 0.33–0.67), sometimes overlapping, and the upper bounds did not include 1.0 (Table 5, Fig. 6). Only with the microsatellite data for White Sisters were there high posterior probabilities ($\bar{x}$ = 0.93) for a single stock origin, the eastern DPS (Fig. 6B). This mixed composition of new

rookeries was also evident in the individual stock proportions presented in Fig. 7, with some animals having higher posterior probabilities of coming from the eastern DPS (blue bars above the abscissa) and others from the western DPS (red bars below the abscissa). The relative contributions of the western and eastern populations to the new breeding sites differed among the two new rookeries with a higher proportion of pups on the Graves Rocks rookery exhibiting western origins while most pups on White Sisters had greater affinity to the east (Figs 3, 5, 6 and 7). The BAYESASS analysis did not yield a consistent pattern of recent migration. Across a wide range of starting parameters, all analyses did identify a close relationship between White Sisters and the eastern population (*m* = 0.146–0.316) and between Graves Rocks and the western population (*m* = 0.188–0.211), but estimates of the magnitude and direction of dispersal between established populations and the new rookeries were unstable (See Supporting information).

Mate choice and genetic introgression on newest rookeries

We used four different approaches to investigate mate choice and genetic introgression on new rookeries. We predicted that no interbreeding between eastern and western sea lions on new rookeries would: (i) result in heterozygote deficits at nuclear markers and (ii) produce pups that had either eastern mtDNA and nDNA *or* western mtDNA and nDNA. As mentioned earlier, the H-W tests on new rookeries did not reveal divergence from random mating expectations (*n* = 30/32 χ^2 tests had *P* ≥ 0.14) that would signify a Wahlund effect (i.e., positive F_{is}) from physical mixing of two populations without interbreeding.

Second, we compared average relatedness, *r*, within groups of pups with 'eastern' or with 'western' mtDNA haplotypes among the established and the new rookeries. For pups with the same maternal lineage, we reasoned that interbreeding on new rookeries might be evident by lower average *r* among pups compared to pups on established rookeries due to a proportion of the former's mothers mating with males from the other population (i.e. mtDNA lineage). Both moment and likelihood estimators of *r* using COANCESTRY did reveal lower average relatedness among pups with 'eastern' haplotypes (and thus from mothers of eastern origin) in the newest rookeries (i.e. White Sisters and Graves Rocks) compared to pups on established rookeries (i.e. Forrester and Hazy, Fig. 8). In many cases, this lower mean *r* was significant (Fig. 9), and the same pattern was observed for a range of baseline allele frequency options. The 'western' pattern was less clear, with many

(A) Established rookeries, *K* = 2
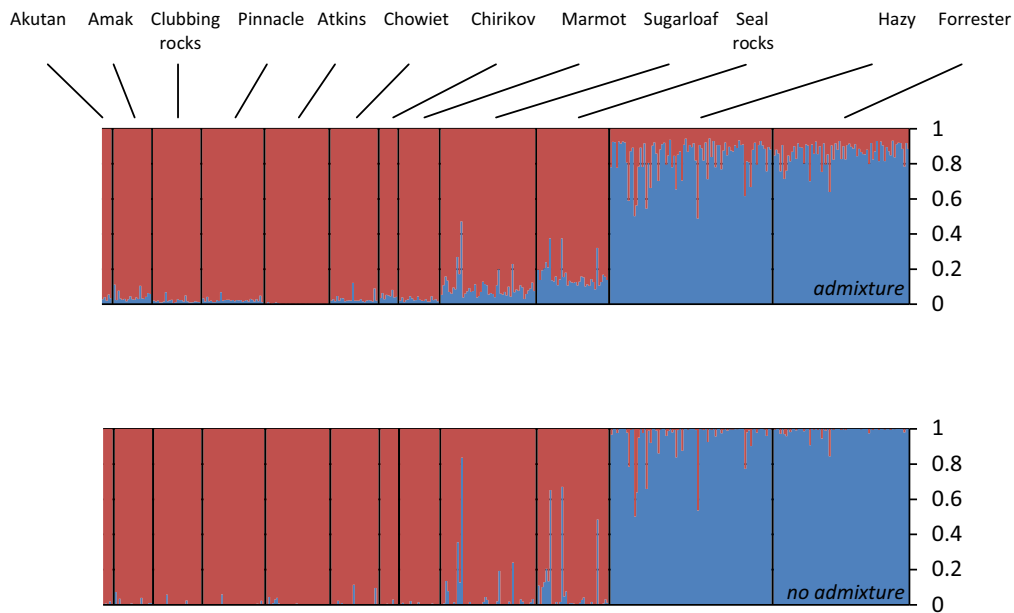
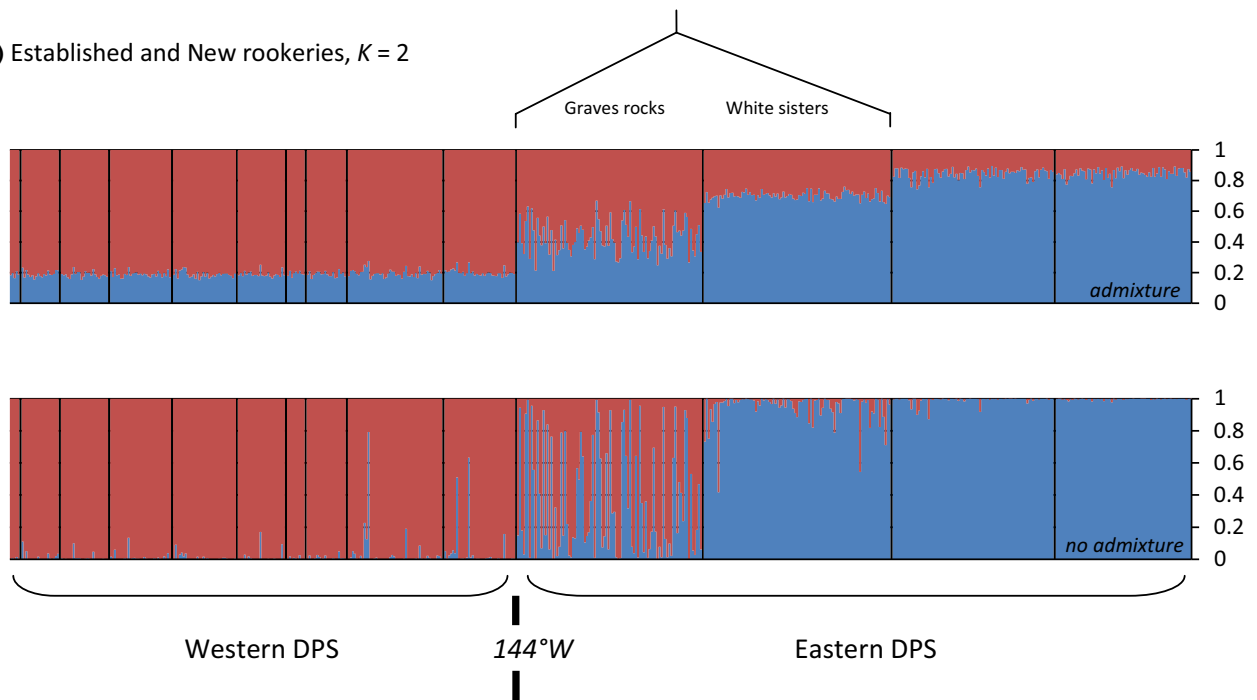

(B) Established and New rookeries, *K* = 2

Fig. 5 Model-based cluster analysis of nDNA data from Steller sea lions using STRUCTURE 2.3.4. (A) Analysis of 399 pups from established rookeries using prior sample group location and both the admixture and no-admixture models of ancestry found *K* = 2 population clusters as the most consistent with the data. (B) Analysis of 588 pups from both established and new rookeries under the same analysis conditions. Each individual is represented by a vertical line with estimated membership, Q, in each cluster denoted by different colours.

western rookeries having very low levels of average relatedness to begin with, possibly indicating greater gene flow among these rookeries.

Third, we examined the STRUCTURE analysis for evidence of mixed ancestry within pups on the new rookeries. Individual assignments identified several pups in

Table 5 Stock proportions of Steller sea lion pups on two new rookeries, White Sisters and Graves Rocks, based on a mixed-stock analysis using BAYES. Parameters of the posterior distributions combined across chains are presented. The second halves of three chains were combined to represent a total 2250–2358 draws from the posterior distribution

Rookery	Marker type	Stock (DPS)	Mean	SD	Posterior quantiles			MCMC
					2.5%	Median	97.5%	
White Sisters	mtDNA	Eastern	0.641	0.061	0.517	0.641	0.755	2250
		Western	0.359	0.061	0.245	0.359	0.483	2250
	nDNA	Eastern	0.931	0.056	0.795	0.942	0.999	2358
		Western	0.069	0.056	0.001	0.058	0.205	2358
Graves Rocks	mtDNA	Eastern	0.329	0.058	0.222	0.326	0.448	2250
		Western	0.671	0.058	0.552	0.674	0.778	2250
	nDNA	Eastern	0.371	0.072	0.234	0.370	0.514	2358
		Western	0.629	0.072	0.486	0.630	0.766	2358
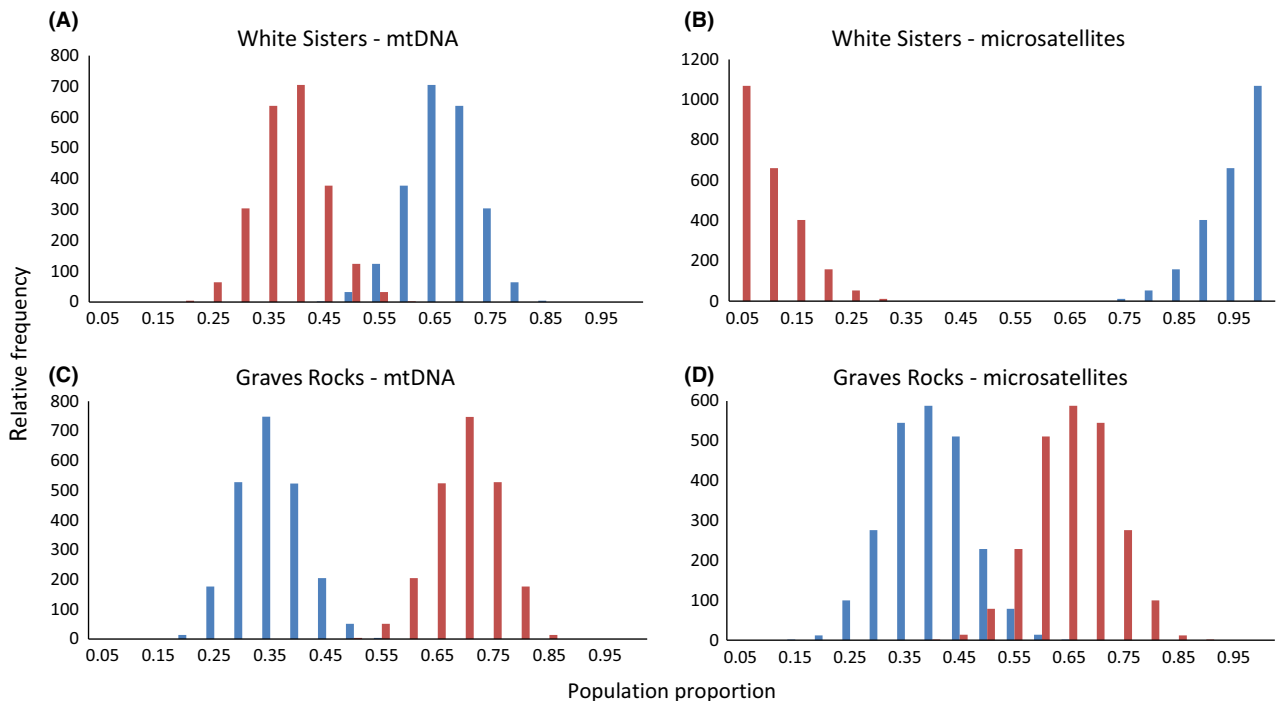
Fig. 6 Posterior distributions of the proportions of two Steller sea lion stocks, the eastern DPS (in blue) and the western DPS (in red) in two new rookeries, White Sisters (A and C) and Graves Rocks (B and D), from a stock-mixture analysis in BAYES for mtDNA (A and C) and microsatellites (B and D).

both rookeries that had high likelihoods of shared ancestry from the east and west population (Fig. 5). Finally, the BAYES analysis, while confirming the presence of eastern and western sea lions on White Sisters and Graves Rocks (see above), also provided evidence of interbreeding among eastern and western immigrants to these new rookeries. Several pups had mtDNA characteristic of one population (dark shaded bars) but nDNA genotypes more characteristic of the other (light shaded bars, Fig. 7).

Discussion

This study demonstrated that the genetic profiling of pups on breeding rookeries could reconstruct the recent demographic history of the dynamic boundary region between two evolutionary distinct metapopulations of Steller sea lion, including the source and pattern of colonization of new rookeries and the mating behaviour of colonists. Many of the study's findings were counter to predictions about dispersal and mate choice based on
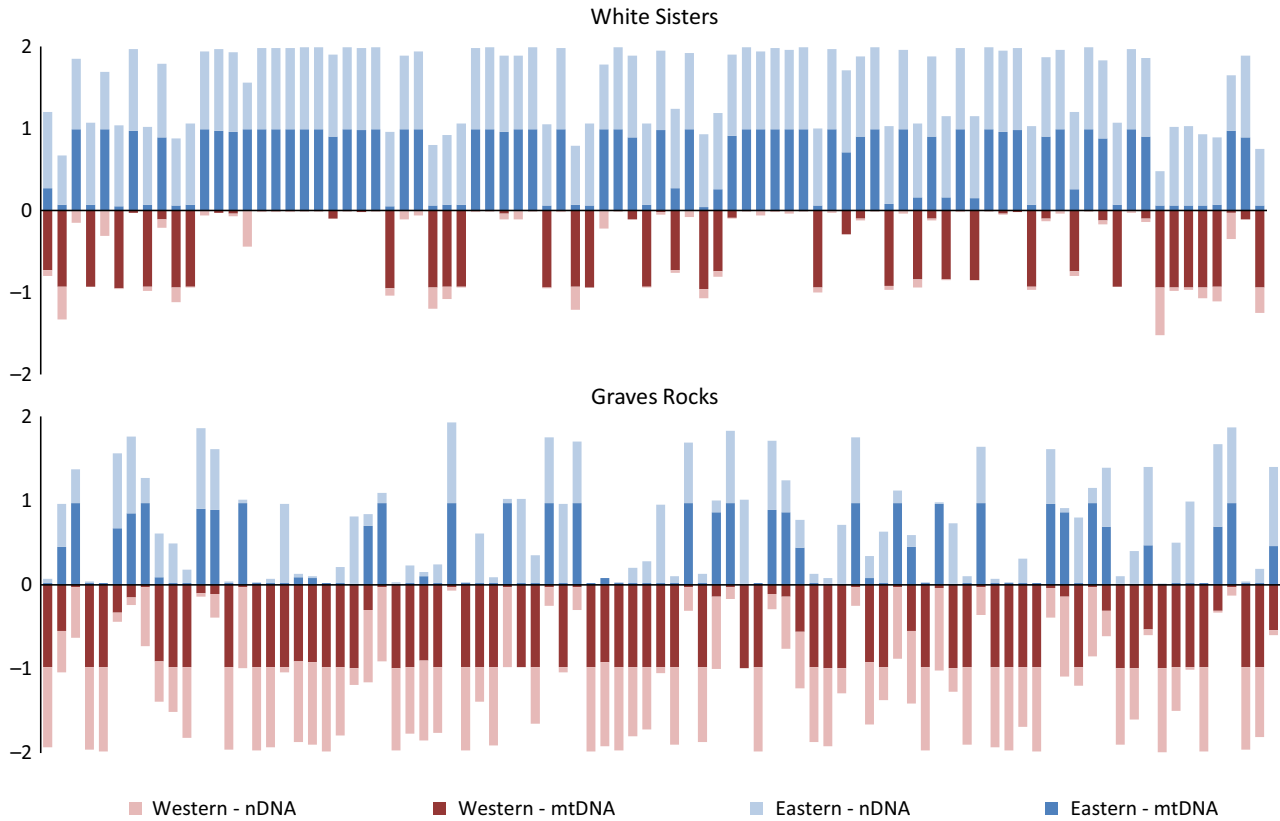
Fig. 7 Assignment of individual pups from White Sisters and Graves Rocks to the eastern (blue) and western (red) DPS based on stock-mixture analysis in BAYES. Findings are presented for mtDNA (dark shading) and multilocus genotypes (light shading) as stacked bars with bar height representing the proportion of times an individual was assigned to each baseline, a value near 1 (or −1) indicating high posterior probability of the pup's haplotype or genotype originating from just one population.

density dependence, inbreeding avoidance and assortative mating involving two presumptive subspecies. These findings have wider implications regarding the diversity of individual dispersal, settlement and mating strategies in both depleted and increasing populations including the colonization of new areas and the recovery of depleted populations and thus have broad import to the management and conservation of species at risk.

The macrogeographic structure recorded in this study supports earlier reports of a long-established division between eastern and western populations of Steller sea lions (Bickham *et al.* 1996, 1998; Baker *et al.* 2005; Harlin-Cognato *et al.* 2006; Hoffman *et al.* 2006; O'Corry-Crowe *et al.* 2006; Phillips *et al.* 2011) and indicated a long history of largely independent population dynamics. The increased resolving power of the present study, however, (i.e., larger *n*, more loci), combined with extensive sampling of recently colonized rookeries and the use of different methods of analysis provided more detailed and unique insights into dispersal and settlement over ecological timescales, especially in the dynamic population boundary region.
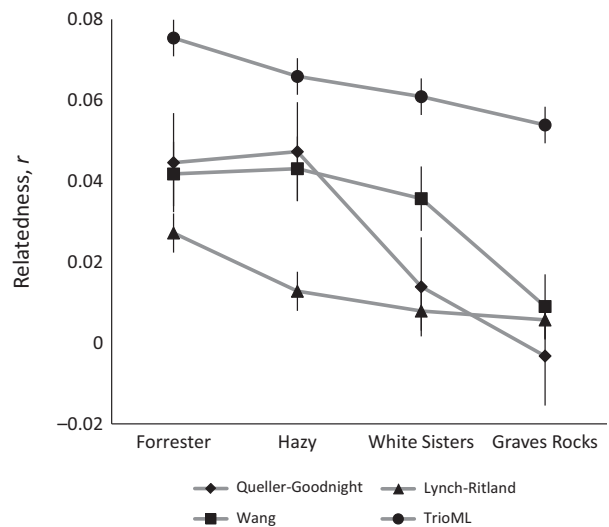

Fig. 8 Average relatedness among Steller sea lion pups with 'eastern' mtDNA lineages on established and new rookeries within the eastern DPS. Estimated relatedness from one likelihood method (TrioML) and three moment methods (Queller-Goodnight, Wang and Lynch-Ritland) using the program Coancestry are presented.
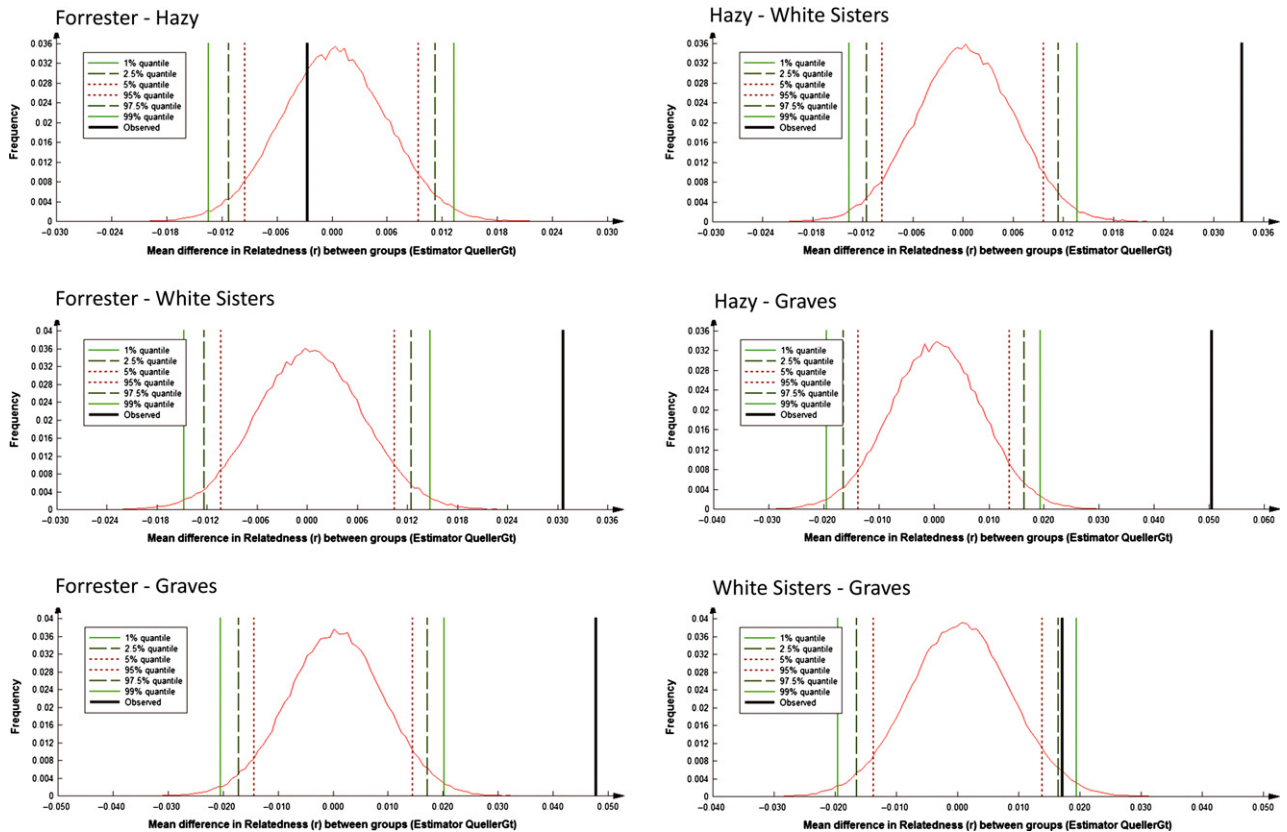
Fig. 9 Mean differences in relatedness among Steller sea lion pups with 'eastern' mtDNA lineages on new compared to established rookeries. The estimator of relatedness, *r*, presented is that of Queller & Goodnight (1989). Significance was assessed via bootstrap (100 000 reps) analysis, the distribution of which is in red. An observed difference in mean *r* (indicated by the black line) is adjudged significant if it occurs outside the confidence intervals (demarcated in green and/or hatched lines) of the null.

The clear break between the two populations contrasts with lower genetic subdivision observed among long-established rookeries within populations, some over 1700 km apart (Table 4). We had previously shown, for example, that there was little evidence of a relationship between mtDNA genetic distance (F_{st}) and geographic distance among western rookeries on the continental shelf ($r^2 = 0.05$, $P = 0.06$; O'Corry-Crowe *et al.* 2006). This was confirmed here for nDNA indicating greater dispersal and gene flow at the local level over time and is consistent with findings that Steller sea lions branded as pups on established rookeries disperse primarily over limited spatial scales (Raum-Suryan *et al.* 2002), and with the characterization of the eastern and western populations as distinct metapopulations (York *et al.* 1996; Raum-Suryan *et al.* 2002).

Against this backdrop of two evolutionarily distinct metapopulations with differing population trajectories, the sequence of new rookery emergence within the intervening distributional hiatus in recent decades is consistent with a stepwise expansion of the growing eastern population under a model of positive density-dependent emigration. The discovery that the newly colonized rookeries were of mixed origin, colonized by sea lions from both the depleted western population and the increasing eastern population, was unexpected. Further, the genetic analysis documented interbreeding and resolved patterns of mate choice on the new rookeries, quantifying the relative contribution of the western and eastern population to each rookery. Investigating the likely causes, mechanisms and consequences of dispersal and colonization in the boundary region leads to the proposition that Steller sea lions from the western and eastern population employed different dispersal and settlement strategies when colonizing the same area in response to sets of local conditions that have made sustained contact relatively rare over time.

Causes of emigration from the eastern and western populations and colonization of new rookeries

Dispersal patterns and range expansion, while primarily driven by habitat quality and competition for resources,

are also shaped by social factors and kinship (e.g. inbreeding avoidance and cooperation), predation, the Allee effect, and by physical and behavioural characteristics limiting dispersal distance (Perrin & Malazov 1999; Perrin & Goudet 2001; Stamps 2001; Clobert *et al.* 2004; Armitage *et al.* 2011). Theoretical and empirical studies have found that species may employ flexible dispersal strategies when colonizing new areas, including both negative and positive density-dependent emigration and settlement (Altwegg *et al.* 2013) and variable expansion rates in relation to habitat quality (Andersen *et al.* 2004).

Population growth in southeast Alaska is expected recovery from over-hunting, but also signals adequate food at broad regional scales. However, traditional breeding sites in southern southeast Alaska appear to be nearing capacity (currently at ∼ 6000 pups/year) with growth slowing dramatically in recent years (Pitcher *et al.* 2007; Hastings *et al.* 2011; Fritz *et al.* 2013). Furthermore, breeding females need to forage regularly prior to pupping and during lactation and so must find food close to the rookery to minimize time spent on foraging trips (Merrick & Loughlin 1997). Dispersal from such sites thus may reflect crowding and local competition for resources (breeding sites, food).

For western sea lions, availability of breeding sites is not limiting. Conversely, availability of quality food resources may be limiting (NRC 2003). Thus, emigration from the areas of decline may be primarily driven by searches for more productive habitat and so may have a positive density-dependent element where declining patch quality has increased local competition. Negative density dependence may also be at play where social factors in a colonial breeder promote greater emigration at lower densities (Altwegg *et al.* 2013).

Although dispersal from the eastern and western populations was likely motivated by the deterioration of local conditions, colonization of new rookeries in northern southeast Alaska was likely driven by improving conditions within this region. The emergence of the new breeding sites coincided with an overall increase in sea lion numbers in northern southeast Alaska at all times of the year that has been linked to improving habitat due to recent (post 1750) glacial retreat and increased productivity of the marine ecosystem (Matthews *et al.* 2011). Higher birthweight, body condition and survival rates of sea lions born on the new rookeries compared to established rookeries in southeast Alaska (Hastings *et al.* 2011) further exemplify the advantages of establishing a breeding site in this region. While avoidance of consanguineous mating may yet be found to influence dispersal in some individuals, the lack of deviation from random mating expectations suggests that inbreeding avoidance was not a primary

motivation for cocolonization of new breeding sites. Similarly, there was no evidence of an outbreeding bias that would indicate positive assortative mating among the recently described western and eastern forms. This is not surprising considering the polygynous mating system of many otariids may facilitate interbreeding among different species (Lancaster *et al.* 2007). Mate quality, however, may be a factor in choosing to colonize a new area. Addressing this question will require teasing apart the genetic and ecophenotypic aspects of lifetime reproductive success and pup survival.

Mechanisms of Steller sea lion dispersal and colonization

Mechanisms of dispersal and settlement often vary with sex, age, social and reproductive status, and individuals may use a variety of strategies to evaluate habitat suitability when assessing the potential costs and benefits of dispersing and where to settle (Greenwood 1980; Perrin & Malazov 2000; Danchin *et al.* 2001; Doligez *et al.* 2002, 2003; Clobert *et al.* 2009). The maximum distance among sea lion rookeries may be determined by how far dispersers are willing to travel before settlement. Although sea lions can undertake movements in excess of 1000 km away from their natal rookery, documented dispersal of marked individuals among established rookeries tends to be restricted to within 500 km of natal site (Raum-Suryan *et al.* 2002; Burkanov & Calkins 2008; Jemison *et al.* 2013). Likewise, there may be limits to colonization distance.

Rookeries within the eastern and western populations are rarely more than 250 km from their nearest neighbour ($\bar{x}$ = 116 km), facilitating individual and genetic exchange. By contrast, for much of the 20th century, the distance between the nearest rookeries in the eastern (i.e. Forrester) and western (i.e. Seal Rocks) populations was over 1000 km (Fig. 1). The primary breeding ranges were even further apart as pup counts were low on peripheral rookeries in both populations for much of this time (Rowley 1929; Mathisen & Lopp 1963; Calkins & Pitcher 1982). The emergence of Forrester as a major rookery mid-century followed by the colonization of Hazy Island, White Sisters and Graves Rocks by the late 1990s closed the gap to 630 km. While the timing of western versus eastern colonization of the two newest rookeries is unknown, the sequence of rookery formation, and the predominance of western lineages on Graves Rocks and the reverse on White Sisters indicate that regional philopatry likely influences colonization behaviour. Spatial clustering of new breeding sites around established ones has been observed in a growing population of New Zealand fur seals, *Arctocephalus forsteri*, where younger animals may choose to establish

breeding colonies close to experienced breeders (Bradshaw *et al.* 2000).

The colonization of just a few rookeries by dispersers from both populations indicates that suitable breeding habitat is limited. Conspecific attraction has been postulated to explain immigration to established rookeries in Steller sea lions (Raum-Suryan *et al.* 2002) and may be a cue for settlement in emerging breeding sites too. Although the presence of conspecifics may signify a breeding site, it does not provide a comprehensive assessment of habitat suitability for successful reproduction. There is growing theoretical and empirical support for the use of public information in breeding habitat selection, where individuals efficiently prospect breeding sites and integrate multiple aspects of habitat suitability (habitat quality, mate quality and availability, kin competition, density dependence, predation pressure, etc.) by assessing the reproductive success of others (Danchin *et al.* 2001; Doligez *et al.* 2004a,b). The ranging capabilities of Steller sea lions, their seasonal and colonial breeding on a limited number of sites, and the spatial heterogeneity in habitat suitability, pup condition and survival suggest that the performance as well as presence of conspecifics may be cues Steller sea lions use to prospect for and assess breeding site quality.

Consequences of recent rookery colonization in northern southeast Alaska

The colonization of new rookeries by eastern and western sea lions in the distributional break between the two DPSs has immediate implications for population subdivision and dynamics. The impact of these colonization events will depend on the intrinsic growth rate of the new rookeries and on the evolving relationship between these sites and their source populations. Pup production on the newest rookeries increased steadily since initial colonization and is now on a par with several rookeries in the Gulf of Alaska (western DPS) but lags behind that at established rookeries in southern southeast Alaska (eastern DPS) (Pitcher *et al.* 2007; Fritz *et al.* 2013). Mark-resight studies recently recorded cross-border immigration to the new rookeries as well as movements between established rookeries in the east and west (Jemison *et al.* 2013). Significantly, perhaps, few movements from the new rookeries back to established rookeries have been observed to date (Jemison *et al.* 2013).

There may also be immediate evolutionary consequences. The greater birthweight and higher survival rates Hastings *et al.* (2011) found in pups born on Graves Rocks and White Sisters, compared to Hazy and Forrester Islands, were considered to reflect a more pro-

ductive, less crowded and safer ecosystem in northern southeast Alaska. Our finding that many of the pups on the newly colonized rookeries in northern southeast Alaska were of western origin indicate that pup size, condition and survival may also have a genetic component. Differing selective pressures shaping size and growth in pups in each population may be manifest on the new rookeries. Furthermore, increased survival rates may reflect greater fitness of pups of mixed genetic origin (e.g. heterosis, heterozygote advantage). Future research will focus on this. Evolutionary consequences will also depend on how frequent sustained contact has been over time. The phylogeographic signal in both mtDNA and nDNA indicates that the mixing and interbreeding of the western and eastern metapopulations documented in this study is a recent and likely rare event. It may be that a wide expanse of unsuitable habitat separated both metapopulations throughout much of their history. This spatial divide, in combination with local conditions within each metapopulation that slowed dispersal and population expansion (e.g. optimal habitat, moderate population density relative to K, philopatry), may have made the sustained contact between east and west that would facilitate genetic introgression rare. All evidence indicates such conditions are not prevailing today.

Management implications

Secondary contact between geographically isolated populations can create unique challenges for stock delineations and the assessment of cross-boundary dispersal. The original Steller sea lion DPS boundary was placed in the then distributional hiatus separating the western from the eastern metapopulations, somewhat arbitrarily at 144°W (Loughlin 1997; Fig. 1). This single meridian is widely taken as the population boundary in population assessment. Recent mark-resight studies and aerial surveys documented movements of both western and eastern sea lions into northern southeast Alaska and characterized these movements as primarily western animals moving east (Fritz *et al.* 2013; Jemison *et al.* 2013). Our genetic findings could be characterized in the same way (Figs 3 and 5). In reality, sea lions from the two populations have cocolonized the habitat that once separated them. Almost certainly not yet at equilibrium, it is hard to predict the future ecological and population dynamics of this newly colonized region or relationship of these sea lions with either source population. Fitting this dynamic mixing zone into one or other management unit, therefore, has risks. It also highlights the limitations of current criteria used in stock delineation where changing spatial and demographic relationships among population segments over

ecological time frames due to range expansions, contractions and distributional shifts are not adequately accommodated.

Conclusions

This study demonstrates that despite apparent ancient separations between geographically distinct populations, changing conditions can result in contact, mixing and even interbreeding among such populations, often in unexpected ways. It informs us that even in cases of population decline and geographic isolation, species may possess the capacity for both positive and negative density-dependent emigration and immigration and that individuals can traverse long distances to settle in new habitat and can successfully interbreed with conspecifics from evolutionarily distinct populations. The study also revealed that the geographic location of unoccupied productive habitat and suitable breeding sites relative to the ranges of extant populations can play a significant role in the pattern of dispersal and colonization that may ultimately result in a major shift in the metapopulation dynamics and genetic structure of the entire species. These findings have broader application to the conservation of species at risk. While the cocolonization of the boundary region may create challenges for the delineation of management units, it also highlights the importance of facilitating long-distance dispersal, the scope and importance of which is often underestimated (Trakhtenbrot *et al.* 2005), and the colonization of new habitat for species recovery. Our findings also revealed that simple models based on positive density-dependent dispersal may be inadequate when assessing range expansion. Finally, the investigation demonstrated the importance on incorporating as many aspects of the behavioural ecology of the species into recovery strategies, where, for example, the successful colonization of new habitat may depend as much on the presence and performance of conspecifics as on ecological assessments of habitat suitability.

Acknowledgements

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Data accessibility

All 130 unique Steller sea lion mtDNA haplotype sequences have been archived on GenBank with accession numbers KM463123–KM463252. These sequences as well as all microsatellite genotype and mtDNA haplotype frequency data have been archived on the Dryad Digital Repository as data package 10.5061/dryad.g6 h52.

Supporting information

Additional supporting information may be found in the online version of this article.

Appendix S1. Model-based cluster analysis of nDNA data from Steller sea lions using STRUCTURE 2.3.4. Analysis of 399 pups from established rookeries in the Eastern and Western DPS (with admixture model of ancestry) that did not use prior

sample group location and found *K*=2 population clusters as the most consistent with the data. Each individual is represented by a vertical line with estimated membership, Q, in each cluster denoted by different colors'.

Table S1. Genetic estimates of recent immigration into Steller sea lion populations.

Table S2. Genetic estimates of recent immigration into two Steller sea lion populations and two new rookeries.

Table S3. Genetic estimates of recent immigration into two Steller sea lion populations and two new rookeries. Details of Table layout and run conditions as in Table S2.