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Mitochondrial DNA perspectives on the introduction and spread of wild pigs in California

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Domestic pigs were first introduced to California by Spanish explorers and missionaries in the 1700s and were soon established as feral populations in coastal regions. Feral pigs are currently abundant in mainland California where their presence in 56 of the state's 58 counties is ecologically problematic. We used molecular techniques to inform on an incomplete record of human introductions associated with escape or purposed release of domestic and European-type wild swine in California, and to provide insight on the mechanisms that produced an accelerated expansion dynamic in the state after the 1970s. We developed mtDNA sequence data for 151 tissue or blood samples from wild pigs spanning their distribution in California, and a 550 base pair segment of the control region was used in phylogenetic analysis. Phylogenetic analyses included our data and 904 published sequences for wild and domestic swine from elsewhere in the U.S. and around the world. Gene flow indicative of natural spread in California was assessed from population-level mtDNA sequence relationships for five population groupings, and we assessed mtDNA haplotypes associated with different periods of invasion by partitioning samples originating from "Historic" occupied counties (before 1968), and "Recent" occupied counties. Nine mtDNA haplotypes were identified among wild pigs California, including three that were unique to California, three that were common elsewhere in the United States, two that were known from Hawaii or other Pacific Islands, and one that was known only from Kentucky, USA. Apparent gene flow between Recent and Historic ranges indicated that pigs dispersed at the regional level, and we identified evidence for expansion by anthropogenic and natural processes from presence of several haplotypes only in Recent range. MtDNA sequence data provided new insight on wild pig expansion in California, including evidence that contemporary translocations promoted hybridization and subsequent spread by natural population growth. Considered together, information on the distribution of common and unique haplotypes and gene flow suggests that range expansion by wild pigs in California is progressing by natural and human-facilitated dispersal, and new introductions from outside of the state. We advocate against additional anthropogenic movement of wild pigs within the state or from other U. S. states because these animals are known to be detrimental to native plants and animals in California ecosystems.

Key words: genetics, mtDNA, haplotypes, sequence, feral pigs, translocation, expansion, invasion, native ecosystems

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Domesticated swine (*Sus scrofa*) were first introduced to North America during the 1500s, and feral pigs were present in wildlands shortly thereafter. Feral pigs had spread into at least 13 southern tier U.S. states by the late 1800s (Mayer and Brisbin 1991), when interest in hunting in the 1900s led to the importation and anthropogenic dispersal of Eurasian wild boar from Europe, which hybridized with existing feral pigs in many parts of the country (Mayer and Brisbin 1991). The consequence was the expansion of European wild pig hybrids and feral-domestic swine from 17 to 44 U.S. states within the last 35 years (Gipson et al. 1998, McCann et al. 2014). Swine is a generic term for all types of pigs, but terms used by resource managers, hunters and the public to refer to feral pigs, European wild boar, and wild pig hybrids vary locally and regionally in the U.S. For clarity, we provide a narrative overview of relevant terminology for domestic, feral, and European wild swine (Appendix I).

The story of wild pigs in mainland California parallels their continental history. Some domestic pigs that were initially introduced by Spanish explorers and missionaries in coastal regions in the 1500s became feral when they were allowed to forage in the oak woodlands (Barrett and Pine 1980). Subsequent additional releases and escape of domestic pigs from livestock pens led to feral populations in other mainland locations by the early 1900s (Pine and Gerdes 1973). Then, in 1925, Eurasian wild boar hybrids (n =12) from Hooper Bald, North Carolina, were released in Monterey County (Pine and Gerdes 1973). These hybrids dispersed and bred with feral pigs, whose progeny were later translocated to other counties in California (Mayer and Brisbin 1991).

Domestic swine were also brought to the Channel Islands by the Spanish in the 1600s, initially on Santa Cruz Island in association with a Spanish penal colony (Sweitzer 1998). Spanish settlement may have also been the source for feral pigs on nearby Santa Rosa Island (Collins 1981). Reliable records document that a small number of feral pigs were translocated from Santa Rosa Island to Santa Catalina Island in the 1930s (Schuyler et al. 2002). At one time feral pigs occurred on five of California's Channel Islands, but all have since been eradicated (Long 1993, Lombardo and Faulkner 2000, Schuyler et al. 2002, Ramsey et al. 2009).

The anthropogenic dispersal of Eurasian wild pig hybrids likely explains recent range expansion in mainland California based on the hypotheses that hybrids may be more invasive than feral-domestic pigs, and also because ranch owners with managed hunting preferentially selected pigs with wild boar characteristics for translocation to their properties (Barrett 1977). Wild-living pigs (hereafter wild pigs) were designated game mammals in California in 1957, after which many private landowners introduced wild pigs to their properties for the purpose of fee hunting (Fitzhugh and Loomis 1993). The popularity of wild pigs as a game species increased in the 1970s and 1980s (Barrett 1993), coincident with concern over increasing ecological and agricultural impacts as they became more widespread. In response, in 1992 the California legislature required hunters to purchase wild pig tags to hunt the species, and a portion of the funds that were generated was directed to research on wild pig biology and their ecological impacts (Waithman et al. 1999, Sweitzer et al. 2000, Sweitzer and Van Vuren 2002).

The combination of wild pigs being included in the Annual Hunter Game Take Survey and records from tag returns revealed that wild pigs expanded in range from nine counties in the 1960s to nearly the entire state by 2006 (Loggins 2007). It remains unclear to what extent natural dispersal, anthropogenic transfer, and the types of breeds of domestic or Eurasian wild pig hybrids that were introduced shaped the expansion and current distribution of wild pigs in California. In the absence of complete records, and considering the clandestine nature of many introductions, molecular techniques provide the best opportunity for examining these factors (Spencer and Hampton 2005).

Mitochondrial DNA (mtDNA) has been used successfully for a number of phylogeographic studies of pigs. Previous work has evaluated the adaptive radiation of suids across Eurasia and identified centers of domestication (Giuffra et al. 2000, Luetkemeier et al. 2010). Published mtDNA sequences have become a valuable resource for global analysis of pig ancestry and dispersal patterns (McCann et al 2014), and several prior investigators used mtDNA to identify ancient anthropogenic dispersal of Eurasian wild boar and domestic swine (Fang and Andersson 2006, Vigne et al. 2009, Scandura et al. 2011). There are known intrinsic limitations of mtDNA sequence data regarding stochastic variation and nuclear pseudogenes (Parr et al. 2006), but these factors do not seem to impact overarching phylogenetic relationships of introduced suids (McCann et al. 2014).

In an analysis of mtDNA from 81 wild pigs collected in 30 U.S. states, McCann (2012) clearly identified the translocation of Eurasian wild pigs from Hooper Bald, North Carolina to California. We therefore considered that a regional analysis of mtDNA sequences within California could provide important perspective on the role of Eurasian wild pigs in recent range expansions within California. Here, we (1) evaluate mtDNA variation in California wild pigs; (2) identify mtDNA haplotypes associated with historic and recent (post 1960s) pig expansion; and (3) evaluate likely mechanisms associated with recent invasion.

MATERIALS AND METHODS

We obtained tissue samples from 151 wild pigs in 23 California counties from 1996 to 2010, spanning recent and historic distribution of the species in the state (Figure 1). We also obtained published mtDNA sequences of wild and domestic swine from 29 other U.S. states, and four other continents (Figure 1). Wild pig tissue samples from throughout California were obtained from USDA Wildlife Services, National Park Service, California Department of Fish and Game, and private organizations involved in sanctioned pig control or eradication programs. For each animal, field personnel collected blood or other somatic

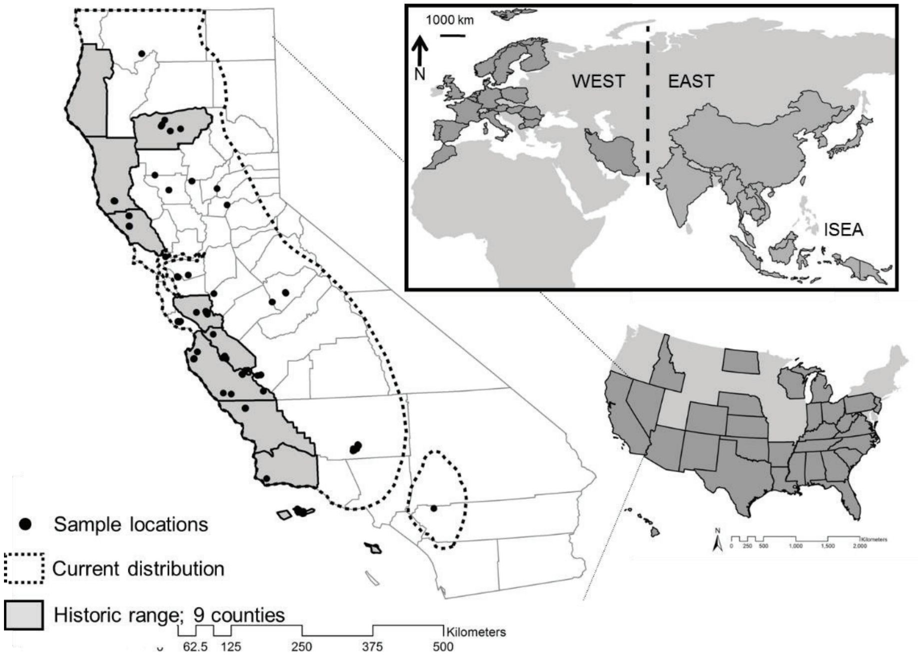

Figure 1.—Distribution of 158 wild pig samples from 23 counties in California, USA, collected from 1996 to 2010 and spanning historic and current invasive range of the species (SCWDS 2010). World geographic location of samples reported among published sequences (*n* = 904) is represented by country (shaded) in inset, including Western Eurasian (WEST) and Eastern Eurasian (EAST) phylogeographic split, and Island Southeast Asia (ISEA) phylogeography denoted for some wild *S. scrofa* and other *Sus* species (*n* = 5). Note: Australia, Hawaii, Iceland, and some Pacific Islands are not shown. United States geography for other published wild pig samples is included.

tissue, recorded pelage characteristics, date, and geographic coordinates for each sample. Blood was transferred to FTA cards (Whatman Inc., Florham Park, NJ, USA), air dried, and stored at room temperature, whereas somatic tissue was frozen and stored at -20°C.

We processed sample specimens using standard protocols as previously described by McCann et al. (2014). We used forward primer PigF (5'-ACTCTGGTCTTGTAAACC-3') and reverse primer PigR (5' –TAAGGGGAAAG ACTGGGC-3') to amplify and sequence an ≈550 base pair segment of the mtDNA control region (Okumura et al. 1996, Loggins 2007). We then submitted sequences to online holdings at NCBI Genbank (http://www.ncbi.nlm. nih.gov/). Accession numbers are as follows: AY96871-AY968729, AY968731-AY968742, AY968744-AY968763, AY968765-AY968806, AY973042, JF702003-JF702008, JF702013-JF702016, JF702018- JF702022, JF702038-JF702039, JF702041-JF702048, JF702050-JF702053, JF702055, JF702079-JF702080, JF702082-JF702104, and JQ792040.

We manually aligned and trimmed sequences for wild pigs from California to match a 403 base pair matrix of 81 mtDNA haplotypes identified for *Sus scrofa* sampled from 30 U.S. states and 904 published sequences for wild and domestic swine from around the world, including Eurasia and Island Southeast Asia (Figure 1; McCann et al. 2014). We included sequences for five other species of *Sus* (*n* =13) in the alignment to serve as outgroups. We collapsed sequences to haplotype using TCS 1.21 with gaps set as fifth character state (Clement et al. 2000). We used JMODELTEST to determine the most appropriate evolutionary model for phylogenetic analysis (GTR+I+G; Guindon and Gascuel 2003, Posada 2008). We then constructed phylogenies in MRBAYES 3.1.2 (Ronquist and Huelsenbeck 2003) with 10,000,000 Markov Chain Monte Carlo (MCMC) sampling generations and retained consensus tree structure with >75% posterior probabilities. To determine if observed mtDNA variation increases with additional regional sampling in California, we compared the number of observed haplotypes for the 151 newly evaluated individual pigs to those previously identified for seven pigs from California (McCann et al. 2014). We then combined all California samples, resulting in 158 individual specimens for analysis of mtDNA relationships.

We identified mtDNA haplotypes associated with different stages of invasion by comparing their geographic distributions to written histories of introduction (summarized by Mayer and Brisbin 1991) and with other evidence for wild pig mtDNA relationships elsewhere in the U.S. (McCann et al. 2014). To facilitate interpretation of molecular data we grouped the 158 California wild pigs into four discrete clusters of geographic importance: (1) Santa Catalina Island ($n = 6$); (2) Santa Cruz Island ($n = 11$); (3) historic mainland ($n = 77$); and (4) recent mainland ($n = 64$). We divided mainland samples by "Historic" and "Recent" distributions, and designated Historic as those samples obtained from nine coastal counties occupied by pigs as of 1967 (Waithman et al. 1999). Recent included animals from all other mainland locations in California (Figure 1).

We assessed gene flow among wild pig clusters in California by analyzing population-level mtDNA sequence relationships in ARLEQUIN 3.5 (Excoffier et al. 2005). We compared five population groupings: the four California populations described above, and an arbitrarily assigned population of wild pigs ($n = 74$) sampled from 29 other U.S. states (29US). We performed a standard analysis of molecular variance (AMOVA) on pair-wise differences with 1,023 permutations to estimate significance. We conducted an exact test of population differentiation with 100,000 MCMC steps based on haplotype frequencies. We then calculated pair-wise F_{ST} and Nm (gene flow) among groups and evaluated results in context of haplotype distributions in California and other states.

RESULTS

We identified 151 haplotypes among all mtDNA sequences, including nine among wild living pigs in California. Phylogenetic analyses revealed three major clades among the 151 mtDNA haplotypes that were associated with geography: Eastern Eurasia (EAST), Western Eurasia (WEST), and Island Southeast Asia (ISEA) (Figure 2). Geography agreed with phylogeny for some groupings, and most haplotypes observed in California were globally distributed and associated with unresolved assemblages in WEST and EAST (M1 and M2, respectively), representing a mixture of domestic swine and Eurasian wild pig hybrids (Figure 2). Three of the nine haplotypes identified for California wild pigs were exclusive to that state (h149, h150, h151), two (h84 and h13) were also found among wild pigs in Hawaii or Pacific Islands, one (h38) matched other continental U.S. wild pigs found only in Kentucky (Table 1), and the other three (h17, h19, h37) were common across the continental U.S. with wide distributions (Table 1). Haplotypes h37, h84, h149, and h151 were confined to single population groupings, and h150 was the only haplotype shared between island and mainland locations (Figure 3, Table 1). Four California haplotypes (h17, h19, h37, h38) were associated with the M1 "mixed" assemblage of wild boar and domestic breeds in WEST, one haplotype (h13) was associated with the M2 mixed assemblage in EAST, and

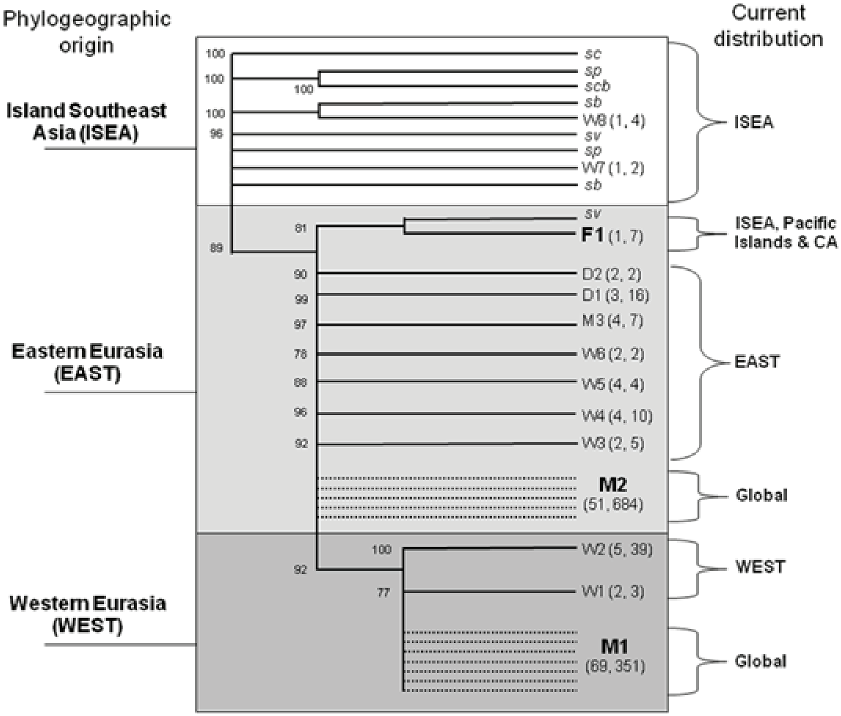

Figure 2.—Cladogram of 151 *Sus scrofa* mtDNA haplotypes constructed for 158 individual pigs from 23 California counties, 74 individuals from 29 other U.S. states, 904 published sequences for *Sus scrofa* from around the world, and 13 sequences from five other *Sus* species for an outgroup. Shaded regions of tree denote overarching phylogeographic associations of mtDNA matching descriptions at left. Current distribution of the haplotypes is denoted by bracketed descriptions at right. Phylogenetic assemblages in tree are described by pig type within EAST and WEST in tree; W (Eurasian Wild Boar), D (domestic), M (mixed, shared between domestic pigs and Eurasian Wild Boar), and F (feral). The five other *Sus* species are abbreviated; *sb* (*S. barbatus*), *sc* (*S. celibensis*), *scb* (*S. cebifrons*), *sp* (*S. philippensis*), and *sv* (*Sus verrucosus*). Groups containing haplotypes found in California are in bold. Dotted lines in the tree indicate polytomies from unresolved phylogenetic assemblages; number of lines is proportional by an approximate factor of ten to the count of haplotypes in each branch. Numbers at nodes indicate mean posterior probabilities (≥75% shown) and numerals in parentheses indicate number of haplotypes and number of individual sequences, from left to right.

one (h84) was linked to the monophyletic F1 group that includes feral pigs observed only in ISEA and on Pacific Islands (Table 1, Figure 2).

In California, WEST mtDNA was more common than EAST (Table 1). Two WEST haplotypes in particular, h17 (n = 59 individuals) and h19 (n = 68 individuals), had wide distributions spanning both Historic and Recent pig range (Figure 3). WEST h150 was found on both Santa Cruz Island and Santa Catalina Island (representing Historic range on the Channel Islands), as well as in Recent range in mainland California (Figure 3). WEST h37 was found in Historic range only, whereas EAST h13 was in Recent range only (Figure 3). Haplotypes h149, h150, and h151 grouped with WEST, but were found only in California.

Table 1.—California geography and breed details for nine mitochondrial DNA haplotypes identified among 158 wild pigs sampled in California from 1996 to 2010. Phylogeographic "Origin" descriptors EAST, WEST and ISEA correspond to a division of mtDNA lineages in Eurasia and elsewhere identified through analysis of a ~400 base pair region of the mtDNA control region. Descriptors M1 and M2 represent unresolved assemblages of mixed domestic and Eurasian wild boar with WEST or EAST phylogenies, and F1 represents feral type pigs from a single ISEA haplotype found only on Santa Catalina Island, Hawaii, and South Pacific Islands. More details are provided by McCann et al. (2014).

Haplotype (Origin)[1]	California region	Phylogenetic group	No. pigs in California	In other US states?[2]	Breed information for haplotype
h13 (EAST)	Recent	M2	8	Yes, HI only	~16 Asian and European domestic breeds
h17 (WEST)	Historic, Recent	M1	59	Yes, six	Linked to Hooper Bald Eurasian wild pig hybrids, and 12 European domestic breeds
h19 (WEST)	Historic, Recent	M1	68	Yes, sixteen	>20 European domestic breeds
h37 (WEST)	Historic	M1	1	Yes, ten	12 European domestic breeds, 1 Asian wild
h38 (WEST)	Historic, Recent	M1	2	Yes, KY only	9 European domestic breeds
h84 (ISEA)	S. Catalina Island	F1	3	Yes, HI only	Feral pigs only
h149 (WEST)	Historic	M1	1	No	Unique to California
h150 (WEST)	S. Catalina and S. Cruz Islands, Recent	M1	15	No	Unique to California
h151 (WEST)	Historic	M1	1	No	Unique to California

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[1] h13 was also in China, Korea, Germany, Spain, Japan, Hawaii, Australia, Thailand, Italy and the United Kingdom; h17 was also in Belgium, Germany, Iberian Peninsula, Iceland, Italy, Macedonia, Norway, Spain, and the United Kingdom; h19 was also in Corsica, Finland, France, Germany, Hungary, Holland, Iberian Peninsula, Italy, Portugal, Sweden, and the United Kingdom; h37 was also in Austria, Bulgaria, Germany, Hungary, Iberian Peninsula, Indonesia, Italy, Korea, Morocco, Poland, Portugal, Spain, and the United Kingdom; h38 was also in Austria, Corsica, Denmark, France, Germany, Hungary, Iberian Peninsula, Italy, Portugal, Sardinia, and the United Kingdom; h84 was also in Papua-New Guinea and Vanuatu.

[2] h17 was also among pigs in AR, MS, NC, ND, NV, and TN; h19 was also among pigs in AL, AR, CO, FL, GA, HI, KY, LA, MI, ND, OH, OK, TN, TX, WI, and WV; h37 was also among pigs in AZ, KS, LA, ND, NJ, NE, NM, OK, PA, TX, and VA.

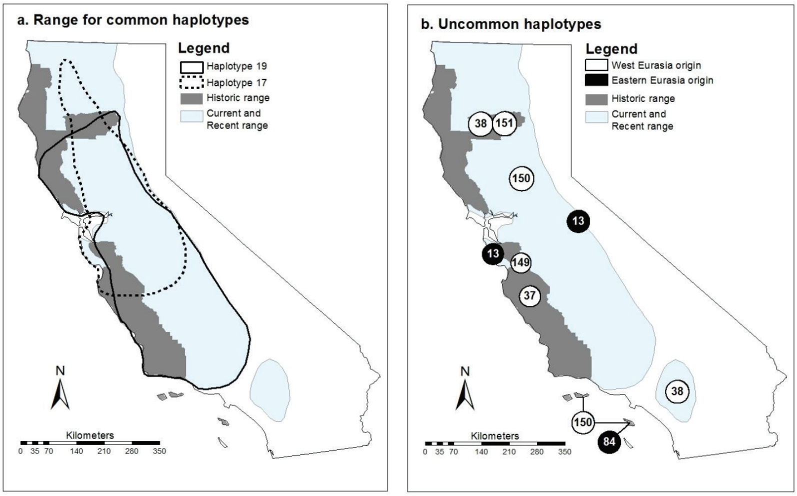

Figure 3.—Distributions of nine mtDNA haplotypes identified among 158 wild pigs sampled in mainland and island locations from 23 counties in California from 1996 to 2010. Haplotypes h17 and h19 were common and widespread with estimated ranges outlined in panel a. Haplotypes that were uncommon with limited distributions (panel b) are the numbers in open (WEST origin) or black circles (EAST origin, except h84 with an ISEA origin). Historic range (nine counties) is shaded gray, and Recent range is shaded light blue.

Population-level mtDNA variation between the five population groupings was significant (AMOVA$_{4,\ 228}$; *P* < 0.001). Exact tests of population differentiation were highly significant (α<0.001) for all but Historic-Recent (*P* = 0.002, *SE* ±0 .001) and Santa Catalina Island-Santa Cruz Island (*P* = 0.030, *SE* ± 0.001). All population F_{ST} measures were significant except Recent and 29US, for which numbers of migrants were estimated as exceptionally high when compared to other populations within the state (Table 2).

Table 2.—Population genetic measures of F_{ST} (bottom half matrix) and *Nm* (top half matrix) for five populations assigned by geography and history of invasion: wild pigs from 29 U.S. states (29US; *n* = 74), historic mainland (Historic; pigs from mainland sites within nine counties of historical occurrence in California; *n* = 77), SCI (Santa Cruz Island California; *n* = 11), SCAT (Santa Catalina Island; *n* = 6), and recent mainland (Recent; pigs from mainland California sites other than the nine historical counties of occurrence; *n* = 64). Significance of *F* statistics between populations is denoted in the lower half of the matrix as follows: **P*<0.05, ***P*<0.01, ****P*<0.0000.

	29US	Historic	SCI	SCAT	Recent
29US	0	4.72864	2.03882	1.71671	98.47133
Historic	0.09563***	0	1.01018	0.33321	8.44260
SCI	0.19695*	0.33109***	0	0.44595	2.65806
SCAT	0.22556*	0.60009***	0.52857*	0	1.07818
Recent	0.00505	0.05591***	0.15833*	0.31682***	0

Discussion

Our phylogeographic result of EAST, WEST, and ISEA groupings agrees with results of other investigators evaluating the mtDNA control region in pigs (Alves et al. 2003, Gongora et al. 2004, Larson et al. 2005), which increases confidence in the accuracy of our dendrogram of sequence relationships associated with wild pigs in California (Figure 2). The unresolved nature of M1 and M2 haplotypes within respective WEST and EAST branches of the phylogeny likely represents an increased rate of nonsynonymous changes in the mtDNA genome resulting from domestication, as described for dogs (Bjornerfeldt et al. 2006). Humans have impacted both the genetic composition and geographic distribution of pigs globally, resulting in a lack of phylogenetic resolution for some mtDNA lineages (McCann et al. 2014). Representatives of M1 and M2 groups with domestic and Eurasian wild pig associations have achieved extensive geographic distributions in North America through anthropogenic dispersal (McCann et al. 2014), and are also prevalent among wild pigs in California (Table 1, Figure 3).

The WEST phylogeographic association for most of the haplotypes (seven of nine) identified in California was not surprising given the major European influence on early settlement of the state. Also, 93% of the 158 wild pigs sampled were linked to a WEST phylogeographic group, including 17 of the 20 feral pig samples from the two islands (Table 1). Geographic distribution of EAST haplotypes was restricted to one island and two spatially separated mainland locations (Figure 3). Both WEST and EAST haplotypes in California were associated with multiple domestic swine breeds, feral pigs, or wild boar from Europe or Asia (Table 1; McCann 2012), but only four of the nine haplotypes were found elsewhere in the continental United States (Table 1). The presence of haplotypes in California from the EAST+ISEA region (Figure 1) indicates anthropogenic transfer of pigs independent of Spanish settlement of California. This result was consistent with an historical account indicating that domestic pigs from the EAST+ISEA region were commonly onboard trade ships that visited coastal regions of California in the mid-1800s (Dana 1840), and sailors likely traded some of those pigs for local supplies prior to return voyages.

Evidence for historic introductions and range expansion.—Although it is not possible to develop a definitive timeline of Historic invasion based upon mtDNA lineages, insights can be gained from assessment of molecular relationships in light of phylogeny and introduction histories. Haplotypes h19, h37, and h38 are associated with a variety of modern domestic breeds (Table 1), which suggests a recent domestic source for these wild pigs in California (Table 1). The lack of current breed references for unique haplotypes h149, h150, and h151 suggests they may represent ancestors of livestock from the Spanish settlement period, and that those mitochondrial lineages are now absent in modern domestic swine. This hypothesis is supported by the distribution of h149, h150, and h151 primarily within the Historic range of pigs in mainland and island locations (Figure 3). Also, the sharing of h150 between Santa Catalina Island and Santa Cruz Island corroborates a common origin for populations on the Channel Islands resulting from introductions by the Spanish (Mayer and Brisbin 1991, McCann 2012).

Spanish settlement likely also explains the occurrence of h150 on the California mainland because there is no record of anthropogenic transfer between island and mainland sources. Notably, h150 was found among wild pigs sampled from the Sutter Buttes in Sutter County, an area identified as Recent expansion range based on the lack of Annual Hunter Game Take survey records before 1967 (Waithman et al. 1999). Of relevance is that the Sutter Buttes region had been privately owned with no public access and no hunting until after 2003 (Sutter Buttes Regional Land Trust 2015), and it is likely that feral pigs were present in Sutter County but not harvested by hunters prior to 1967.

Haplotypes h17 and h19 were the two most common in both Historic and Recent range in California, and their current distributions likely represent a history of human transfer followed by local population growth and expansion (Pine and Gerdes 1973, Waithman et al. 1999). Haplotype h17 was previously linked to Eurasian wild pig hybrid stock translocated to California from Hooper Bald, North Carolina in 1925 (McCann 2012). The geographic distribution of h17 in California is consistent with the known and documented anthropogenic dispersal of Eurasian wild pig hybrid animals within the state to the north and east of the original introduction site in Monterey County (Figure 3; Mayer and Brisbin 1991). Nevertheless, h17 was also found further to the north, east, and south of areas of known anthropogenic transfer, and it is apparent that once Eurasian wild pig hybrids were translocated outside of Monterey and Tehama counties, they then expanded their distribution into new areas of the state as populations increased (Figure 3).

Haplotype h19 was the most common among the 158 pigs sampled (Table 1), with an expansive coastal and inland distribution from central California to northern California (Figure 3). Haplotype h19 is also widely distributed in 16 other U.S. states with a genetic linkage to >20 different breeds of domestic swine (Table 1; McCann et al. 2014). We therefore conclude that the distribution of feral-domestic pigs with the h19 haplotype in California is indicative of anthropogenic transfer, followed by local expansion resulting from population growth and dispersal. Further we have photographic evidence (pelage characteristics; McCann et al. 2003) that feral-domestic pigs (h19) are interbreeding with Eurasian hybrids (h17) within their broad zone of overlap (Figure 3). This is important because, based on our research here, we now know that wild pigs of all types in California are of mixed heritage (M1 hybrids; Figure 2), and fully capable of invasion and range expansion resulting from vigorous population growth.

Haplotypes h13 and h38 were uncommon in the state and their role in range expansion is less clear. Feral-domestic h13 was the only EAST mtDNA lineage in California, suggesting that it does not link to livestock associated with Spanish settlement (Table 1). Further, h13 was found exclusively within Recent range (Figure 3). Feral-domestic h38 was identified in both Historic and Recent range (Figure 3), and h38 was the only haplotype among wild pigs in a population that was recently established in southern California (Figure 3; detailed by Loggins 2007). These observations indicate that feral-domestic pigs with h13 and h38 Eastern Eurasian heritage were recently introduced to California, either by escape from livestock pens, or purposed anthropogenic transfer from out of state (Table 1). The spatial separation between wild pig sample locations for both h13 and h38 suggests that anthropogenic transfer was the more likely source (Figure 3).

Assessment of population relatedness revealed lack of differentiation for wild pigs in Historic vs. Recent range, coupled with high levels of gene flow between the ranges (≈8 migrants/generation; Table 2). In contrast, there was genetic differentiation and very low gene flow between Santa Cruz Island and Santa Catalina Island (Table 2), consistent with no records of anthropogenic transfer of wild pigs after the 1930s. It is not always possible to separate natural dispersal from anthropogenic dispersal based solely on molecular evidence, but human facilitated gene flow (= anthropogenic transfer) was obvious for haplotypes h13 and h38 based on distance and presence of geographic barriers to animal movement between the sampling locations (Figure 3). Wild pigs also naturally disperse on their own (Waithman et al. 1999), accounting for animals with haplotypes h17 and h19 occurring in proximity within regions (Figure 3).

An important observation for future consideration is that Loggins (2007) surveyed fewer samples for mtDNA analyses, and additional sampling by our work identified presence of several additional mtDNA haplotypes in California. It is therefore possible that expanded sampling in Humboldt County (historically invaded; Waithman et al. 1999) and along the eastern margin of the San Joaquin Valley (Sierra Nevada foothills) would identify presence of previously unknown haplotypes.

Haplotype patterns among feral pigs on the Channel Islands.—We interpret the low mtDNA diversity observed on Santa Catalina Island (two haplotypes) and Santa Cruz Island (one haplotype) as due to genetic drift associated with isolation or bottlenecks from culling and mast failures (Baber and Coblentz 1986). Both populations endured periodic reduction from the 1940s onward, prior to eventual eradication in the early 2000s (Schuyler et al. 2002, Ramsey et al. 2009). The low mtDNA variability on the islands coupled with presence of abundant feral pigs prior to eradication suggests that wild pigs are resilient even at low genetic diversity.

The discovery of h84 on Santa Catalina Island is interesting, as this haplotype has been observed nowhere else except islands elsewhere in the Pacific Ocean (Figure 2; Larson et al. 2005, Loggins 2007). A possible pathway for introduction of h84 to California was the third voyage of Captain Cook from 1776 to 1779 (Cook 1968, Loggins 2007). Cook transferred livestock between many islands in the southern Pacific Ocean and also visited the Oregon coast, although we could find no records of visits to mainland California or the

Channel Islands. Due to the apparent absence of this haplotype on Santa Cruz Island and the lack of samples for Santa Rosa Island (the source of pigs for Santa Catalina Island), the origin of h84 in California remains unclear.

Importance for management and conservation.—Sequence variation in mtDNA among wild pigs provided valuable insights on the introduction history and on the subsequent, but undocumented, translocation and liberation of wild and domestic pigs that occurred after the 1950s (Waithman et al. 1999). Considered together, information on the distribution of haplotypes and gene flow suggests that range expansion by wild pigs in California is progressing on multiple fronts through population growth coupled with natural dispersal, anthropogenic dispersal, and new introductions from outside of the state (McCann 2012). Natural expansion was not limited to Eurasian wild pig hybrids, however. We found strong indications that feral-domestic wild pigs have moved into new areas independent of anthropogenic transfer.

Identification of new mtDNA lineages among wild pigs in California also suggests that public interest in hunting wild pigs continues to shape the molecular profile of California's wild pigs (McCann et al 2014). California state officials suspect recent importation and release of wild pigs from elsewhere in North America (B. Gonzales, California Department of Fish and Wildlife, personal communication), and our results indicate that this very likely has occurred. Considering the pervasive spread of wild pigs already in progress, additional introductions will be detrimental and should be vigorously discouraged to limit the spread of livestock and zoonotic diseases (Benfield et al. 1999, Jay et al. 2007), and to prevent further damage to native plants and animals, which has been focused in California oak woodland ecosystems (Sweitzer and Van Vuren 2002, Cushman et al. 2004, Grinde 2006, Loggins 2007, Wilcox and Van Vuren 2009).

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APPENDIX I: TERMINOLOGY COMMONLY USED FOR WILD AND DOMESTIC SWINE

Swine is a generic term commonly used by livestock producers and many state and federal resource managers for all types of domestic, feral and wild pigs. **Eurasian** or **European wild boar** refers to all wild, non-domesticated pigs, which are the ancestors of most domestic breeds of pigs. **Domestic pigs** are swine that are penned and associated with active animal husbandry. **Feral pigs** are domestic pigs that have escaped captivity in the recent or distant past, are no longer associated with active husbandry, and are descended from domesticated individuals. Feral pigs are also commonly referred to as **feral hogs** or **feral swine**, particularly in the southeastern U.S. and Texas. The terms **razorback** and **wild hog** are American colloquialisms, loosely applied to any type of feral pig, wild boar or wild pig hybrid (Wikipedia 2015).

European wild boar are sometimes called **Russian wild boar**, but the species occurs throughout Europe, and European wild boar is more appropriate. European wild boar freely interbreed with feral pigs wherever populations came into contact, leading to **European hybrid wild pigs** with a range of intermediate phenotypic characteristics (Mayer and Brisbin 1991). Phenotypic characteristics do not reliably identify ancestry, however (Mayer and Brisbin 1991), and genetic analysis is the most reliable method for verifying presence of European wild boar hybrids within feral pig populations (McCann et al. 2014). In areas where there is a known history of introduction and interbreeding of Eurasian wild boar with feral pigs, hybrid wild pigs are often referred to as wild boar, which is technically incorrect from a genetic perspective.

California is one of the regions in the U.S. where wild pigs from North Carolina that were thought to be pure or near pure European wild boar were translocated in the 1920s (McCann et al. 2014), pen reared, and then released into the wild for hunting. Once in the wild, the North Carolina-origin wild pigs interbred with feral pigs where feral pigs were already present (Waithman et al. 1999). Feral and European wild pig hybrids have been managed as a big game mammals in California since 1957 (Sweitzer et al. 1999), and are officially referred to as **wild pigs** by the California Department of Fish and Wildlife. Most hunters, landowners, and the general public also use wild pigs when referring to feral pigs and European hybrid wild pigs in the state. Because free ranging swine in California include a diversity of feral and wild type ancestors, wild pigs is an appropriate term for them.