Protection from harvesting restores the natural social structure of eastern wolf packs

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Abstract

Legal and illegal killing of animals near park borders can significantly increase the threat of extirpation for populations living within ecological reserves, especially for wide-ranging large carnivores that regularly travel into unprotected areas. While the consequences of human-caused mortality near protected areas generally focus on numerical responses, little attention has been given to impacts on social dynamics. For wolves, pack structure typically constitutes an unrelated breeding pair, their offspring, and close relatives, but intense harvest may increase adoption of unrelated individuals into packs. Concerns that high human-caused mortality outside Algonquin Park, Canada threatened the persistence of eastern wolves, led to implementation of a harvest ban in surrounding townships. We combined ecological and genetic data to show that reducing anthropogenic causes of mortality can restore the natural social structure of kin-based groups despite the absence of a marked change in density. Since implementation of the harvest ban, human-caused mortality has decreased ($P = 0.000006$) but been largely offset by natural mortality, such that wolf density has remained relatively constant at approximately 3 wolves/100 km^2. However, the number of wolf packs with unrelated adopted animals has decreased from 80% to 6% ($P = 0.00003$). Despite the high kinship within packs, incestuous matings were rare. Our results indicate that even in a relatively large protected area, human harvesting outside park boundaries can affect evolutionarily important social patterns within protected areas. This research demonstrates the need for conservation policy to consider effects of harvesting beyond influences on population size.

1. Introduction

Conservation and management strategies, including decisions to remove species from endangered lists, are largely based on estimates of population size and sustainable harvest (Pyare and Berger 2003; Whitman et al. 2004; Isaac and Cowlishaw 2004; Patterson and Murray 2008). There is, however, growing evidence that maintenance of family groups within species that exhibit kin-based social structure can have fitness benefits associated with the adaptive evolution of sociality (Pope 2000; Silk 2007; Gobush et al. 2008). Despite the potential importance of kinship, the role of social groups in long-term population persistence is routinely overlooked (Haber 1996). In protected areas, exploitation near park borders further complicate conservation efforts because these edge effects can significantly increase risk of extirpation, especially for carnivores that have large home ranges (Woodroffe and Ginsberg 1998).

In the absence of strong harvest pressure, wolf packs (*Canis lupus*, *C. lycaon* and their hybrids) are typically kin-based (Mech and Boitani 2003). Although some variability in this model has been reported (Meier et al. 1995; Forbes and Boyd 1997), exceptions are rare in naturally-regulated populations. High mortality from hunting and trapping may, however, disrupt this natural social structure by prompting the adoption of unrelated animals into wolf packs (Grewal et al. 2004; Jedrzejewski et al. 2005). Thus, anthropogenic influences may play an important role in the social structure of kin-based species. In fact, due to the high propensity for compensatory demographic responses in wolf populations subject to exploitation (e.g. Fuller et al. 2003; Adams et al. 2008), marked changes in wolf population social structure, including those related to kinship within packs and/or inbreeding, may occur even in the absence of numerical changes. This compensatory paradigm provides an important challenge for the restoration and maintenance of not only viable, but also naturally-functioning, populations where fitness is likely to be optimized when evolutionary adaptation is driven by natural rather than artificial (i.e. human-mediated) selection pressures (Darimont et al. 2009).

The eastern wolf (*C. lycaon*) is designated as a species of special concern by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) under Canada's Species at Risk Act (SARA). One of the largest protected areas (7571 km^2) for eastern wolves is Algonquin Provincial Park (APP) in Ontario, where 200 – 300 resident wolves have been influenced by hybridization with gray-eastern wolf hybrids (*C. lupus x lycaon*) that occur north of the park, and with eastern coyotes (*C. latrans* var.) south and west of the park (Grewal et al. 2004; Wilson et al. 2009). Between 1987 – 1999, eastern wolves in APP suffered high mortality (56-66%) from hunting and trapping when they left the park to hunt deer outside park boundaries (Forbes and Theberge 1996; Theberge et al. 2006). It was speculated that this intense harvest was responsible for low kinship within packs (Grewal et al. 2004) and that extirpation of wolves in APP was likely if human-caused mortality was not curbed (Vucetich and Paquet 2000; Patterson and Murray 2008). In December 2001, due to prevalent concern for the long-term viability of wolves in APP, the Government of Ontario, amidst much public controversy, banned wolf harvest in townships adjacent to APP, thereby increasing the protected area for park wolves by 6340 km^2 (Fig. 1). The purpose of this study was to determine whether wolf pack structure changed in APP following inception of the harvest ban. Specifically, we used previously published data (Grewal et al. 2004) combined with current field data and genetic profiles to test the hypothesis that the ban elicited measurable effects on wolf pack structure. We predicted that extending protection for wolves into areas previously experiencing high human-caused mortality would prompt the renewal of kin-based wolf packs and initiate the restoration of a natural social structure for wolves in APP.

2. Materials and Methods

2.1. Study area

The 2700 km^2 Continuous Study Area (CSA) surveyed consists of rolling hills on the southern margins of the Canadian Shield. The area is forested with pines (*Pinus strobus, P. resinosa, P. banksiana*), shade-intolerant hardwoods (*Acer rubra, Populus tremuloides, P. grandidentata, Betula papyrifera*) and lowland conifers (*Abies balsamea, Picea glauca, P. mariana*). On moister uplands, shade-tolerant hardwoods (*Acer saccharum, Betula alleghaniensis*), along with *Tsuga canadensis* predominate. Lakes, rivers and ponds are common. Although we monitored wolves across the entire park during 2002 – 2007, for comparability we consider here population trend and cause of death data only for an area of eastern Algonquin (881 – 2635 km^2) that corresponded with the previously described CSA (Theberge and Theberge 2004). It should be noted that packs used for pedigree analysis in this study include wolves monitored outside the CSA and therefore the sample size for animals included in the post-ban pedigree analysis (n=138) is higher than that used for the post-ban density and proportional mortality data (n=112).

2.2. Wolf Population Density and Determination of Causes of Death.

Wolf population density within the CSA was estimated during eleven consecutive years prior to the harvest ban (1989 – 1999) using territory mapping as described by Theberge and Theberge (2004). Territories were defined based on 95% minimum convex polygon (MCP; Mohr 1947) to exclude locations resulting from off-territory excursions (Bekoff and Mech 1984; Potvin 1988). The effective sampling area varied annually but averaged ~1250 km^2. For each year's population estimate, a census area was defined by a concave polygon enclosing all adjacent territories within the study area. The total number of wolves (including both territorial and non-territorial animals) was summed in the census area (Messier 1985; Ballard et al. 1987; Fuller 1989) with density (N_t), given as wolves/100 km^2, estimated as the summed maximum pack sizes plus the estimated number of lone wolves in the area, divided by the census area (Mech 1973; Fuller 1989). The number of lone wolves in the area was estimated from the proportion of lone wolves among the radio-collared sample in the study area each year. Confidence intervals are not included with density estimates because they were unavailable for the pre-ban dataset (see Theberge and Theberge 2004).

We employed the same methods described above to post-ban data to estimate wolf density in an area of eastern Algonquin (881 – 2635 km^2) similar to the CSA during winters 2003 – 2007. We radio-tagged 112 wolves within this study area between August 2002 and February 2007 as described by Patterson et al. (2004). Each wolf was fit either with a VHF radiocollar (Holohil Systems Ltd., Woodlawn, Ontario, Canada and Lotek Engineering Inc., Newmarket, Ontario, Canada) weighing approximately 400 g, or Lotek model 4400S or M GPS collars (weighing approximately 500 and 950 g, respectively, Lotek Engineering, Inc., Newmarket, Ontario) that were scheduled to obtain fixes at approximately 90 minute intervals during November – April. Additionally, young pups were manually captured from their natal dens and weighed, sexed, and implanted with a VHF radio-transmitter (2 x 8 cm, Advanced Telemetry Systems, Isanti, MN or Telonics, Inc., Mesa, AZ) in the peritoneal cavity (Crawshaw et al. 2007). All radio transmitters contained mortality switches that doubled the signal pulse rate if the transmitter remained motionless for >7 hours. Wolf capture and handling procedures were approved by the Ontario Ministry of Natural Resources' animal care committee (permit nos. 02-75, 03-75, 04-75, 05-75, 06-75, 07-75).

We checked radio-tagged wolves for mortality signals from the ground or during aerial tracking at <1-2 week intervals throughout the year, and when a mortality signal was detected, we promptly visited the site on the ground. Cause of death for each wolf was determined by assessing evidence at the mortality site and detailed necropsies conducted by personnel from the Canadian Cooperative Wildlife Health Centre, University of Guelph.

2.3. DNA Extraction and Amplification.

Blood samples were collected during radio-collaring activities conducted from August 2002 – January 4, 2007. DNA was extracted from 205 samples; 196 from blood on FTA cards or blood clots and 9 from pulled hair, with a DNEasy Blood and Tissue Extraction Kit (Qiagen, Mississauga, Canada). Of these, 138 were affiliated with packs and were included in kinship analyses. Hair was cut into lengths of approximately 2 cm and placed directly into 500 μL 1X lysis buffer (4 M urea, 0.2 M NaCl, 0.5% *n*-lauroyl sarcosine, 10 mM CDTA (1,2-cyclohexanediamine), 0.1 M Tris-HCl, pH 8.0). Two 6 mm diameter hole punches from the whole blood on FTA paper were placed in 500 μL 1X lysis buffer and then DNA was extracted according to manufacturer's directions. For the blood clots, 350 – 400 mg was removed from the top portion of the clot to increase the chance of obtaining the buffer coat layer where the majority of white blood cells remain after centrifugation. The clot was fragmented with a scalpel blade, placed in 1 mL of 1X lysis buffer in a 15 mL tube, and rotated at 37 °C overnight (12 – 18 hours). A 500 μL subsample of lysate was removed and placed in 1.5 mL Eppendorf tubes. Proteinase K (2.4 Units) was added and samples were incubated at 65°C for 1 hour with pulse vortexing after 30 minutes and at the end of 1 hour. Samples were then transferred to a 65 °C water bath inside a 37 °C incubator for one hour to allow slow cooling to 37 °C, at which time a second aliquot of proteinase K (2.4 Units) was added to each sample followed by pulse vortexing and incubation at 37 °C overnight. A 250 μL subsample was removed and placed in new 1.5 mL Eppendorf tubes. DNA extraction from the blood clots from this point on was according to manufacturers directions. All samples were quantified with Picogreen^{TM} (Molecular Probes) (Ahn et al. 1996) and subsequently diluted to 2.5 ng/μL. For those samples below the threshold of 3 ng/μL, the undiluted extract was used in PCR and quantified by gel fluorescence with ethidium bromide (Ball et al. 2007) to ensure that all samples had between 0.5 – 5 ng of template DNA for each PCR. We amplified a 343 – 347 bp fragment of the mitochondrial DNA control region

(Wilson et al. 2000) to assign maternal haplotypes, a 658 bp section of the Y-intron (Shami 2002) and 4 Y-microsatellites (Sundqvist et al. 2001) to track paternal inheritance, and 16 autosomal microsatellite loci (cxx377, cxx172, cxx123, cxx109, cxx225, cxx250, cxx200, cxx204, cxx147, cxx253, cxx383, cxx410, cxx442, c2010, cph11, c2202) (Grewal et al. 2004) to determine individual genotypes and bi-parental inheritance. Amplified fragments were size-separated and visualized on a MegaBace 1000 (GE Healthcare, Baie d'Urfé, Quebec), sequences were edited in BioEdit 7.0.9 (Hall 2007) and genotypes were scored in GeneMarker 7.1 (SoftGenetics, State College, PA).

2.4. Parentage and Kinship Analysis

All pre-ban data relating to kinship within packs was taken from Grewal et al. (2004). A pack was defined as ≥3 individuals living concurrently within a group. Pack affiliations were determined using multiple telemetry locations and ground tracking, as well as visual observations made during telemetry tracking flights. Specifically, pack affiliations were inferred when the animals in question were located together, within a common territory, during >75% locations over a period extending > 30 days. In two cases (W113/C4361 in McKaskill and W195/C4443 in Pretty; Supplementary Figures 1k, 1n), male individuals unrelated to other pack members were not considered adopted because their presence was confirmed only after contact was lost (due to dispersal, death, or collar failure) with the breeder of the same sex; in such cases we could not rule out the possibility that the new individual was replacing the "lost" animal as the breeder.

Samples genotyped at fewer than 8 loci were not included in the analysis (n = 4, all from hair) to ensure high probability of identity, and an additional 5 samples were excluded because they represented previously sampled animals. A total of 196 animals were included in the parentage analysis; overall, missing data accounted for 1.4% of the dataset. The autosomal microsatellite dataset was assessed for genotyping errors with MicroChecker

(VanOosterhout et al. 2004). To test the power of our dataset for individual identification, we calculated the probability of identity (PID) and probability of identity for siblings (PIDsibs) (Taberlet and Luikart 1999) in GenAlEx 6.1 (Peakall and Smouse 2006). In the parentage analysis, females were excluded as the mother if the mitochondrial haplotype was inconsistent with the putative offspring, and males were excluded as the candidate father of male pups if either the Y-intron haplotype or Y-microsatellites were inconsistent with those of the putative offspring. We then utilized two different methods to assign parents: 1) the exclusion method, considered the "paragon" of parentage analysis (Jones and Ardren 2003) but can result in false exclusions (Pompanon et al. 2005), and 2) a maximum likelihood approach (95% confidence) implemented in CERVUS 3.0.3 (Kalinowski et al. 2007). CERVUS is a robust parentage analysis software package that accounts for rare alleles, genotyping errors, and null alleles by using simulations to statistically assign the most likely parent among all non-excluded parents. Paternity simulations generated 100,000 offspring with 100 candidate males (assuming a park population estimate of 200 animals and a 1:1 sex ratio) and assuming 57% of the population was sampled (based on 57 males ≥1 year sampled over a 5-year period and an average 5-year lifespan) and allowed a standard error rate of 0.010. We used KINSHIP 1.3.1 (Goodnight and Queller 1999) to test the hypothesis that individuals within packs were more likely to be related at the half-sibling and full-sibling level than unrelated based on a simulation series of 10,000 pairs generated from allele frequency calculations of 124 adults (pups excluded). KINSHIP uses relatedness (*r*) values, allele frequencies, and comparative genotypes to calculate the likelihood of the relationship hypothesis being tested. Pairs that were assigned as not significant (based on a critical *P*-value of 0.05) in the test of half-siblings were considered unrelated. Where relatedness was indicated but specific kinship was unclear, we used ML-Relate (Kalinowski et al. 2006), a program that accommodates null alleles and uses simulations and a maximum likelihood approach, to test hypotheses between putative and alternative relationships, to assign the most probable relationships based on 10,000 simulations.

When comparing mitochondrial DNA and Y-chromosome haplotypes (Fig. 3), for pre-ban data (1995 – 2001) all animals sampled in 2 – 5 of 6 consecutive years were included (pups were not sampled from the den); for post-ban data (2002 – 2007) (Figs. 3 and 4) pups sampled from the den were excluded unless they were confirmed in the pack 1 year later.

3. Results

3.1 Wolf Population Density and Causes of Death

In winter 2003, approximately 14 months after initiation of the harvest ban, we estimated wolf density in eastern APP at approximately 3 wolves/100 km^2 (Fig. 2), suggesting an average rate of increase (r_t) = 0.20 between 1999 and 2003. However, no further increases in density were observed between 2003 – 2007 despite a marked reduction in mortality from hunting and trapping within the ban area and park during this period (Table 1; P = 0.000006). This was due in part to natural causes largely replacing anthropogenic causes as the leading mortality agents for wolves following inception of the harvest ban (B.R. Patterson et al. unpublished data; Table 1).

3.2. Parentage and Kinship

The mean level of observed heterozygosity for APP wolves sampled post-ban was high (H_O = 0.687) and similar to the levels of 0.694 – 0.725 reported by VonHoldt et al. (2008) for non-inbred wolves in Yellowstone National Park. Grewal et al. (2004) also reported high levels of heterozygosity during the pre-ban time period, although no estimates were given. No loci showed a significant deviation from Hardy-Weinberg equilibrium after Bonferroni correction. Probability of identity among siblings was low (PID_{sibs} = 1.06 x 10^{-6}) indicating that full-siblings in this group were unlikely to have the same genotype. We created pedigrees for 138 individuals living in 17 packs over a 5-year period (Supplementary Figure

1a-1q). Parent-offspring relationships were identified in all 17 packs, nine of which had both breeders identified. Only one pack (Cauliflower; Supplementary Figure 1e) had an animal that was unrelated to the breeder. This female yearling was the only unrelated adult of the 59 non-breeding adults identified within all of the 17 packs over the 5-year period. Further, this female dispersed in March 2003, the first winter of our study, and subsequently became a breeding female in another territory along the southern edge of our study area. The overall proportion of packs that had adopted unrelated animals (here defined as unrelated at the half sibling level) decreased significantly post-ban (Table 1; P = 0.00003) demonstrating that post-ban packs are less likely to adopt unrelated animals.

We found that incestuous matings were generally avoided despite high kinship within packs. Only two of the 17 post-ban packs had related breeding pairs: one had a half-sibling breeding pair (Beechnut; Supplementary Figure 1b) and another had a full-sibling breeding pair (Louisa; Supplementary Figure 1j). There were three packs in which daughters became subsequent breeders: two while the mother was still in the pack (Cauliflower and Leaf; Supplementary Figures 1e, 1i) and one after the mother dispersed (LaFleur; Supplementary Figure 1h). In one case the full sibling of the breeding male (both unrelated to the breeding female) replaced his brother as breeder while the brother was still in the pack (Jocko; Supplementary Figure 1g). In two cases (W113/C4361 in McKaskill, W195/C4443 in Pretty; Supplementary Figures 1k, 1n) a male unrelated to all others in the pack was identified within the pack after the death of the breeding male, and in one instance (W131/C4379 in Achray, Supplementary Figure 1a) a male unrelated to the breeders was caught in the pack after contact was lost with the breeding male, and in this case the new male became the breeder.

Within 5 packs known to occupy the same territory both prior to, and following, the harvest ban, single mitochondrial DNA haplotypes in females were more common post-ban (Fig. 3). Single Y haplotypes were common during both time periods but where a second Y haplotype was documented in post-ban packs (Pretty and McKaskill) it was found in an unrelated animal caught after the death of the breeding male (Fig. 3). This pattern was similar in the other post-ban packs studied (Fig. 4). In Big Crow, the BB Y-haplotype was found in animals caught after the death of the male with the CG Y-haplotype, and in Sunday the Y-haplotypes represent two pairs of full siblings of which brothers with the BB Y-haplotype were documented in the territory after the brothers with the AA Y-haplotype had dispersed or died (Fig. 4). The C9 mtDNA haplotype in Cauliflower (Fig. 4) represents the one non-breeding adult found that was unrelated to the female breeder in the pack.

4. Discussion

Our results suggest that high levels of hunting and trapping of wolves outside the borders of Algonquin Provinical Park prior to the harvest ban were responsible for the low kinship observed within packs. Extending protection for APP wolves has, therefore, helped restore a more naturally structured population consisting of family-based wolf packs, despite stable wolf densities since implementation of the ban. More specifically, adoption of unrelated animals into packs is almost non-existent in the current population.

The restoration of a family-based social structure in APP, including the pattern of female recruitment from within the pack but acceptance of unrelated immigrant males, presumably as potential breeders after breeder loss, is congruent with naturally-regulated gray wolf populations in park preserves where wolves have legal protection such as Yellowstone National Park in Wyoming (VonHoldt et al. 2008) and the Białowieża Primeval Forest in Poland (Jedrzejewski et al. 2005). Also in concordance with wolf studies in Denali National Park in Alaska, Superior National Forest in northeastern Minnesota, and Yellowstone National Park (Smith et al. 1997; VonHoldt et al. 2008), incestuous matings in

APP were generally avoided despite high kinship within packs. Together, these results indicate that the natural social fabric has been restored for wolves in Algonquin.

Although the long-term viability of APP wolves has been the subject of some debate (see Theberge et al. 2006; Patterson and Murray 2008), we consider the social restoration of pack structure to be a positive response to the harvest ban because it represents an important element of a naturally-functioning ecosystem, the maintenance of which is a primary goal for Ontario Parks, the agency responsible for management of provincial parks; this social component may stimulate natural regulation at other trophic levels. In general, assessments of population viability typically focus on numerical responses and estimates of sustainable harvest (Pyare and Berger 2003; Whitman et al. 2004; Theberge et al. 2006; Isaac and Cowlishaw 2004; Patterson and Murray 2008), with the impacts of human exploitation on social dynamics being largely ignored, even in highly social large mammals such as lions (Whitman et al. 2004) and wolves (Haber 1996). There is, however, growing evidence suggesting that maintaining kin relationships in socially structured populations is evolutionarily important and can have positive effects on fitness (Silk 2007). For example, female red howler monkeys (*Alouatta sericulus*) living in kin-based groups have higher reproductive success than those living in unrelated groups (Pope 2000), and female elephants (*Loxodonta africana*) in well established family groups with old matriarchs have lower levels of stress hormones and higher reproductive output than those in groups that have been socially disrupted by poaching (Gobush et al. 2008). Therefore, focussing solely on abundance when assessing population status may ignore other potentially important factors that can contribute to long-term fitness, and hence persistence, of populations.

Wolves are highly intelligent animals that have evolved under a family-based social framework. Although the influence of this structure on fitness is not well understood, recent work suggests that maintaining the social organization of wolf packs is important for effective resource use (i.e. knowledge of prey distribution and ability to detect, pursue and subdue prey) (Sand et al. 2006; Stahler et al. 2006), pup survival (Brainerd et al. 2008; Schmidt et al. 2008), and may be effective, at least in part, at precluding hybridization with coyotes (*C. latrans*) due to the lower turnover of individuals within packs and the tendency during hybridization events for genes to flow from the more common into the rarer species (Grant et al. 2005). Breeder loss is particularly influential and can result in abandonment of territories, dissolution of social groups, and smaller pack size (Brainerd et al. 2008). Mate loss can also result in unusual behavioural responses of the surviving breeder (Smith and Ferguson 2005) or incestuous pairings if mate loss occurs close to breeding season (VonHoldt et al. 2008).

Minimizing the anthropogenic impact on social structure in populations that form highly related groups is likely to improve overall fitness by allowing evolutionary processes to occur in response to natural selection, not human-mediated mortality (Darimont et al. 2009). In this way, conservation strategies can bolster the adaptive evolutionary potential of populations facing environmental fluctuations, including climate change. When compared to other conservation and management approaches such as translocations and habitat restoration, reducing levels of exploitation by expanding no-harvest zones to include areas outside park boundaries is a relatively simple, long-term solution to promote persistence of top predators that are integral to healthy ecosystems (Terborgh et al. 2001, Soulé et al. 2003, Chapron et al. 2008).

We conclude that the harvest ban around Algonquin has restored the natural social structure of wolf packs in the park. Given the fitness benefits of kin-based groups in animals that have evolved complex social patterns, these results are likely relevant to other socially structured animal populations that experience high human-caused mortality near park borders. Our results demonstrate the need for conservation policies that look beyond numbers to include the subtler, but potentially important, impacts on social dynamics of wildlife. Future work addressing the fitness elements associated with harvesting and the adaptive evolution of family groups will add significantly to our understanding of how centuries of harvesting have shaped the genetic evolutionary potential of *Canis* and other family-based species.

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Table 1. Impact of harvest ban on cause of death[a] and adoption of unrelated wolves into packs[b] in Algonquin Park, Ontario.

Time period	Number (%) of human-caused deaths*	Number (%) of natural deaths	Number of packs with ≥ 3	Number (%) of packs that had unrelated animals**
Pre-Ban	42 (67)	21 (33)	15	12 (80)
Post-Ban	5 (16)	26 (84)	17	1 (5.9)

[a] Data are from radio-collared animals. Pre-ban mortality data are based on an 11-year sampling period (1989 – 1999) (Theberge and Theberge 2004); post-ban mortality data are based on a 5-year sampling period (2002 – 2007). [b] Pre-ban pack data are based on sampling between 1987 – 2001 (Grewal et al. 2004); post-ban pack data are based on sampling between 2002 – 2007. * and ** indicate significance based on a two-tailed Fisher's exact test ($P = 0.000006$ and $P = 0.00003$, respectively).

Figure Legends

Figure 1. Map of study area. Pack territories are fixed kernel home ranges and those outlined in black represent packs compared in Figure 3. Deer wintering areas are occupied by deer in early winter or when snow cover is light and less than 30 cm in depth; deer yards are the core of the deer wintering areas and is used when movement of deer is restricted due to severe weather conditions when snow depth is greater than 46 cm.

Figure 2. Wolf density in Algonquin Park, Canada. Pre-ban (1989 – 1999) data are from Theberge and Theberge (2004). Post-ban data was collected after a hunting and trapping ban was implemented in townships surrounding the park.

Figure 3. Comparison of pre- and post-ban haplotypes. Mitochondrial DNA control region and Y-chromosome microsatellite haplotypes found in packs occupying the same territory during pre- and post-ban time periods. Maternal haplotypes are based on the mitochondrial DNA control region and paternal haplotypes are based on Y-microsatellites. Where different, the pre-ban pack name is included in parentheses.

Figure 4. Maternal and paternal haplotypes in post-ban packs. These packs are in addition to those shown in Fig. 3. * indicates inferred haplotype based on paternity analysis. As in Fig. 3, pups sampled from the den were excluded unless they were confirmed in the pack 1 year later. Two packs (Flat Iron and LaFleur) are not shown because there was only 1 female and 1 male adult representative in the pack.

Supplementary Figures 1a-1q. Pedigrees for 17 post-ban eastern wolf packs in Algonquin Provincial Park, Ontario. Pack names are shown at the top of each pedigree. Pink circles are females, blue squares are males, individuals have a unique identifier made up of the field sample tag (Wxxx) plus a database number (Cxxxx), dashed lines identify additional relationships between individuals, HS = half-siblings, FS = full-siblings, UR = unrelated, ? = unknown individual, check mark indicates those animals that were identified at some point over the 5 year period as a non-breeding adult, triple lines around circle or square indicate an individual that is, for clarity, duplicated in the pedigree, red outline identifies the one individual that was an adopted adult animal that was unrelated to any other animal in the pack, a dashed box outline indicates male animals that were captured after the death of the breeding male or loss of contact with the breeding male. W58 is represented twice in these figures because she dispersed from the Achray territory and subsequently became the breeder in Flat Iron. Sample sizes (n) are indicated for each pack. However, it should be noted that all animals represented in the pedigree were not necessarily alive or located together at the same time because the pedigrees represent up to 5 years of monitoring.

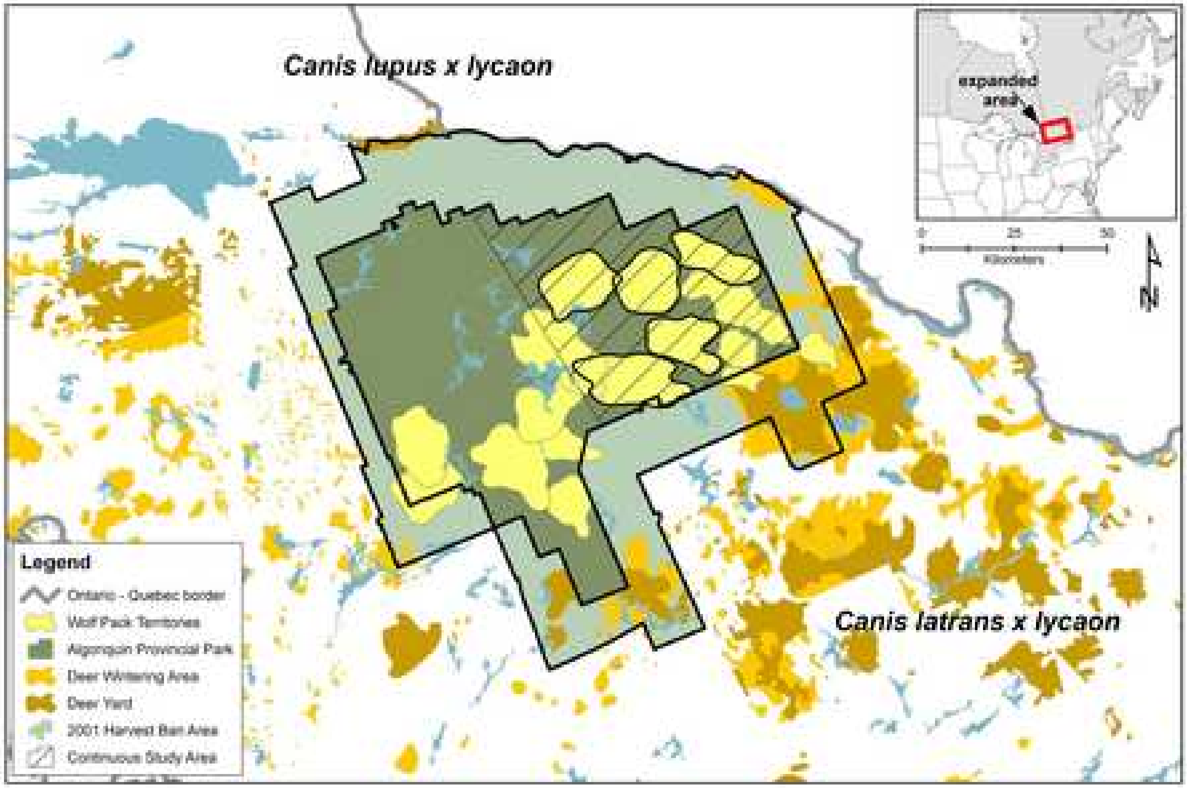

Canis lupus x lycaon
expanded area
0
25
50
Kilometers
N
Legend
Ontario - Quebec border
Wolf Pack Territories
Algonquin Provincial Park
Deer Wintering Area
Deer Yard
2001 Harvest Ban Area
Continuous Study Area
Canis latrans x lycaon

Fig. 1

Figure 2

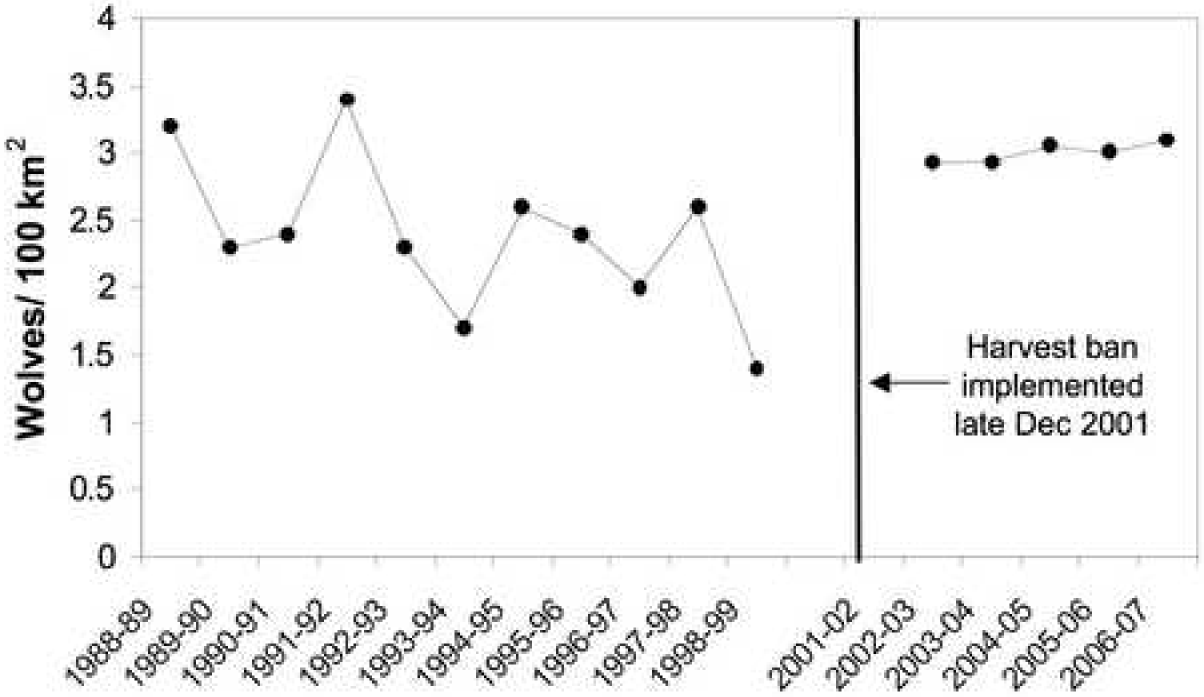


Fig. 2

Figure 3

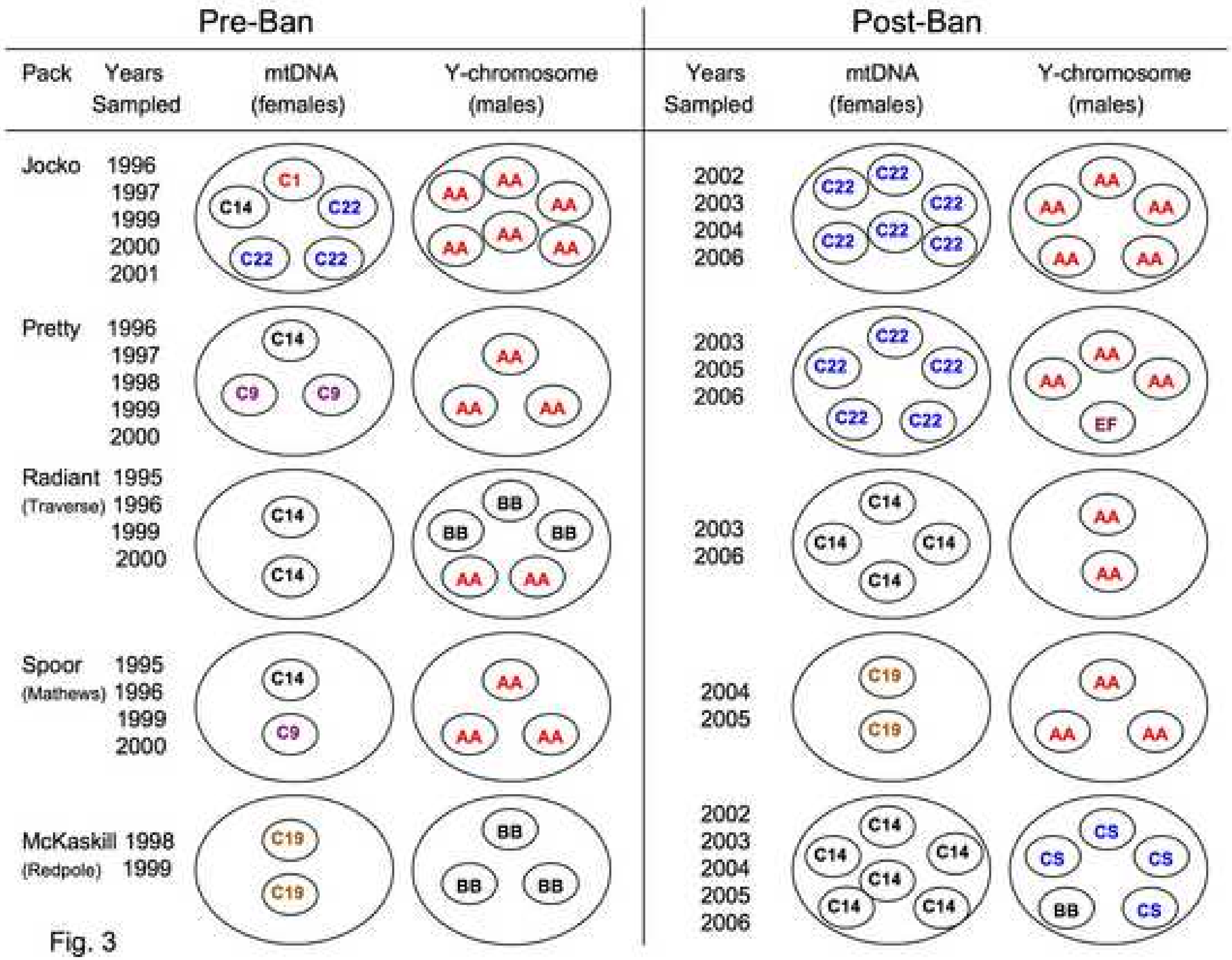
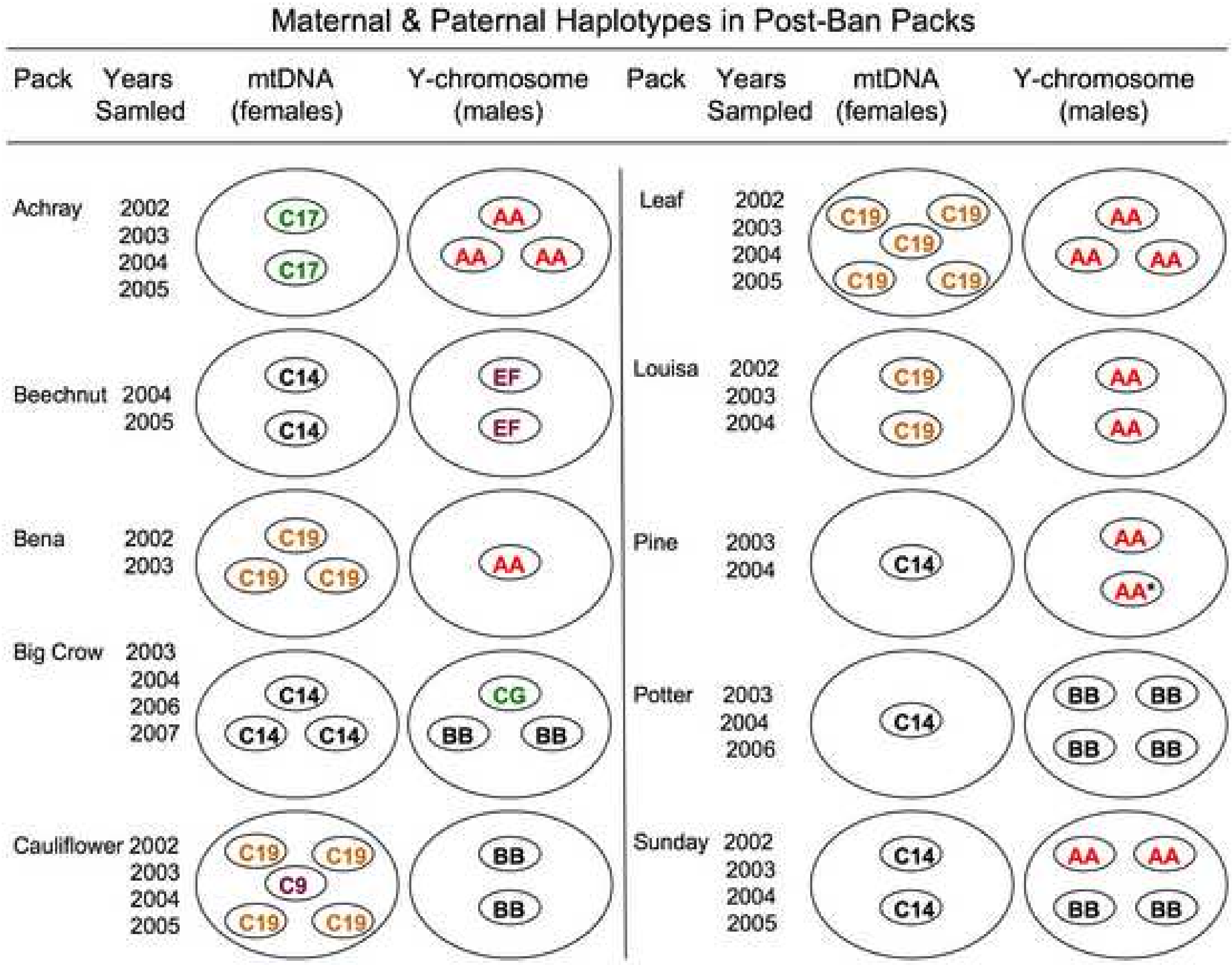


Fig. 3

Figure 4



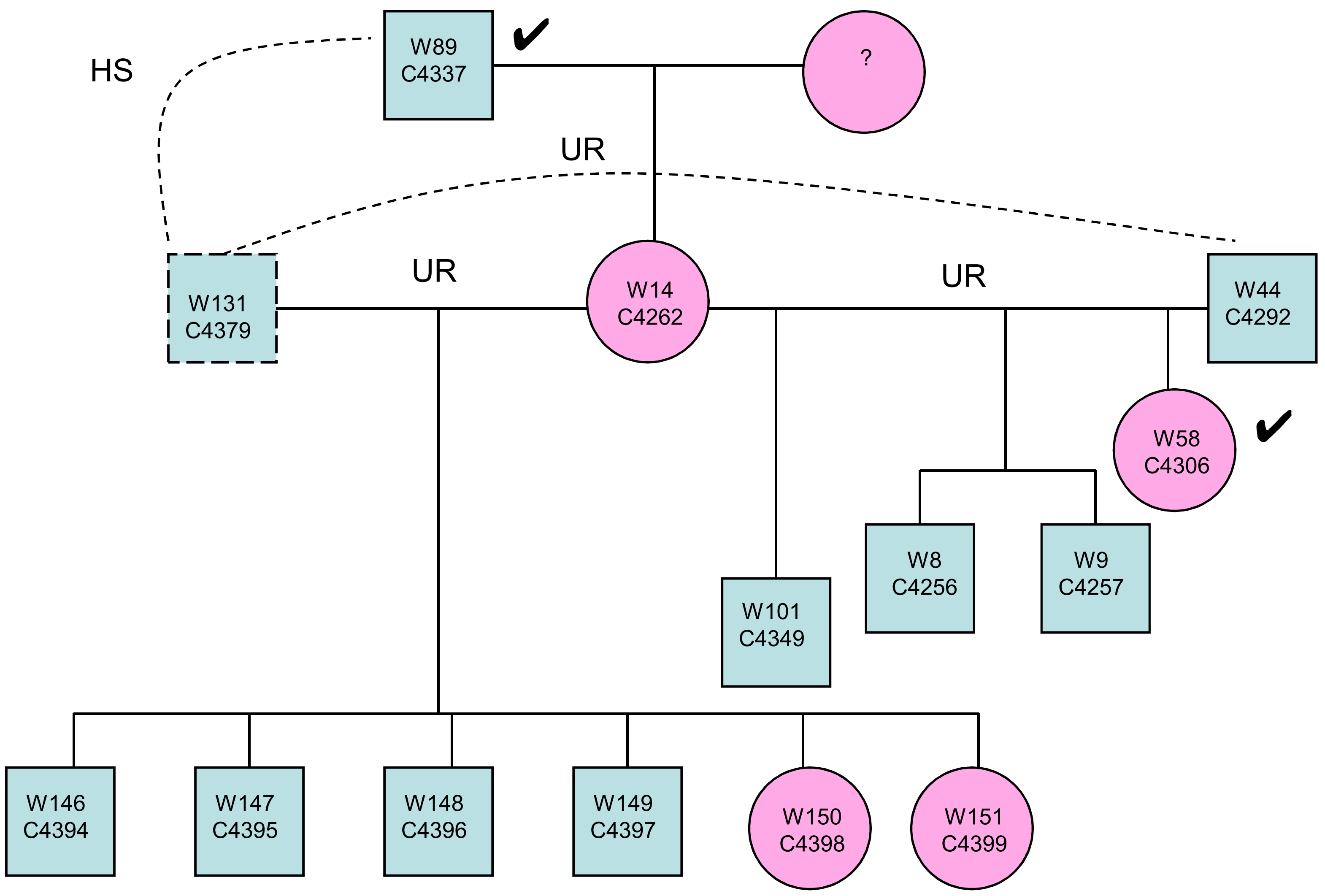
Maternal & Paternal Haplotypes in Post-Ban Packs

Pack	Years Samled	mtDNA (females)	Y-chromosome (males)	Pack	Years Sampled	mtDNA (females)	Y-chromosome (males)
Achray	2002 2003 2004 2005	C17 C17	AA AA AA	Leaf	2002 2003 2004 2005	C19 C19 C19 C19 C19	AA AA AA
Beechnut	2004 2005	C14 C14	EF EF	Louisa	2002 2003 2004	C19 C19	AA AA
Bena	2002 2003	C19 C19 C19	AA	Pine	2003 2004	C14	AA AA*
Big Crow	2003 2004 2006 2007	C14 C14 C14	CG BB BB	Potter	2003 2004 2006	C14	BB BB BB BB
Cauliflower	2002 2003 2004 2005	C19 C19 C9 C19 C19	BB BB	Sunday	2002 2003 2004 2005	C14 C14	AA AA BB BB

Figure 4.

n=14

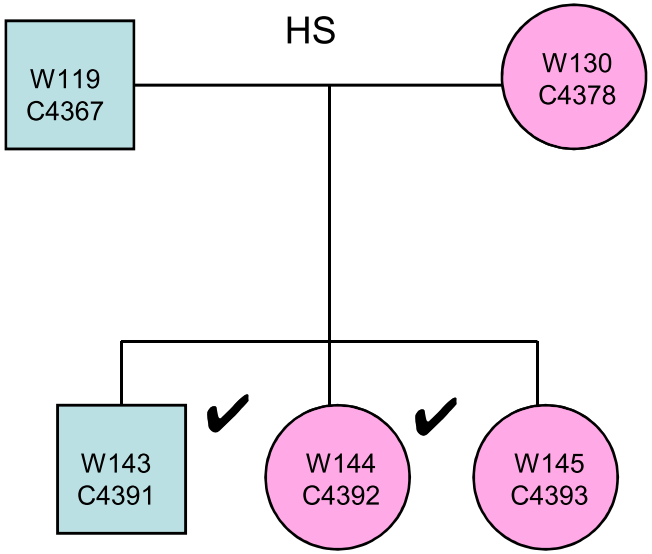
Achray



Supplementary Fig. 1a

n=5

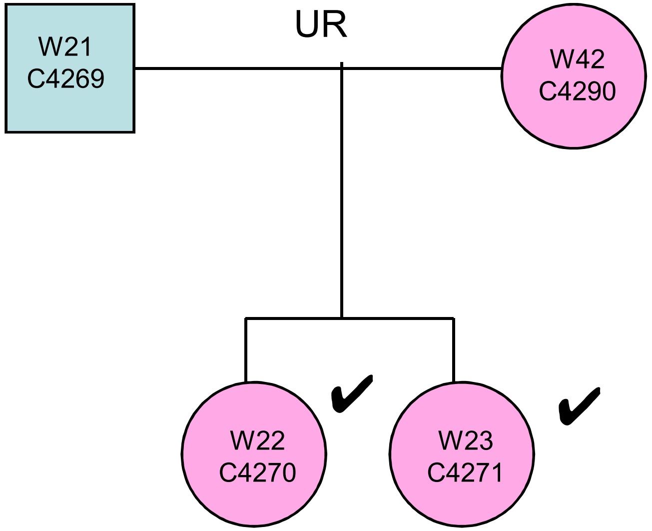
Beechnut



Supplementary Fig. 1b

n=4

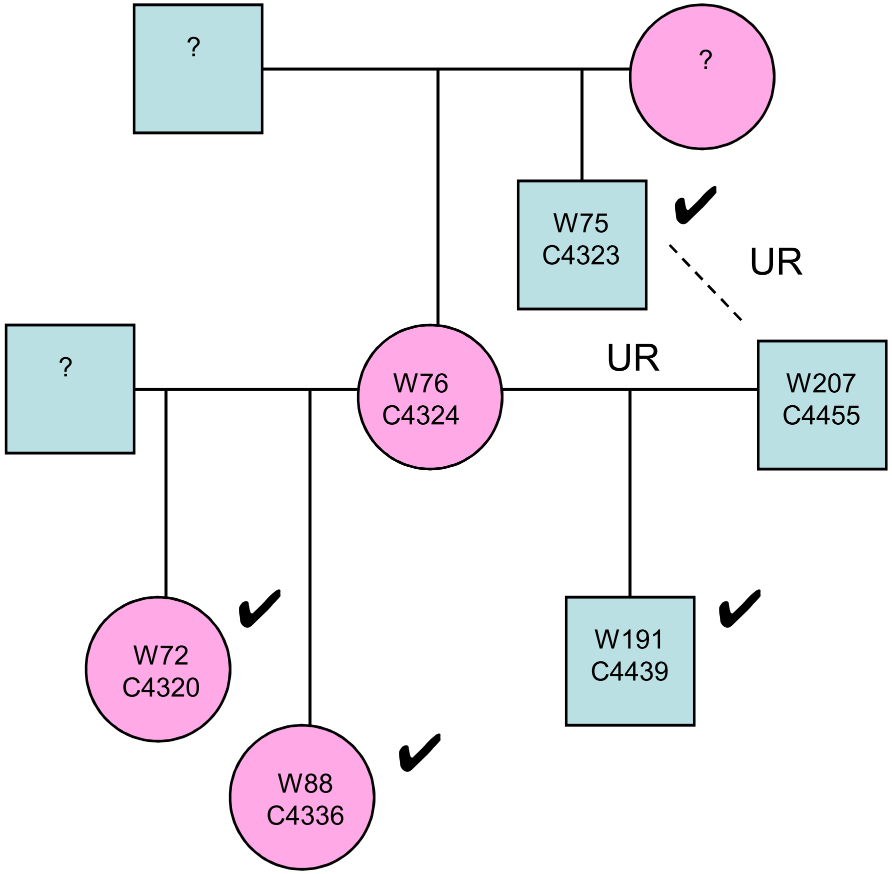
Bena



Supplementary Fig. 1c

n=6

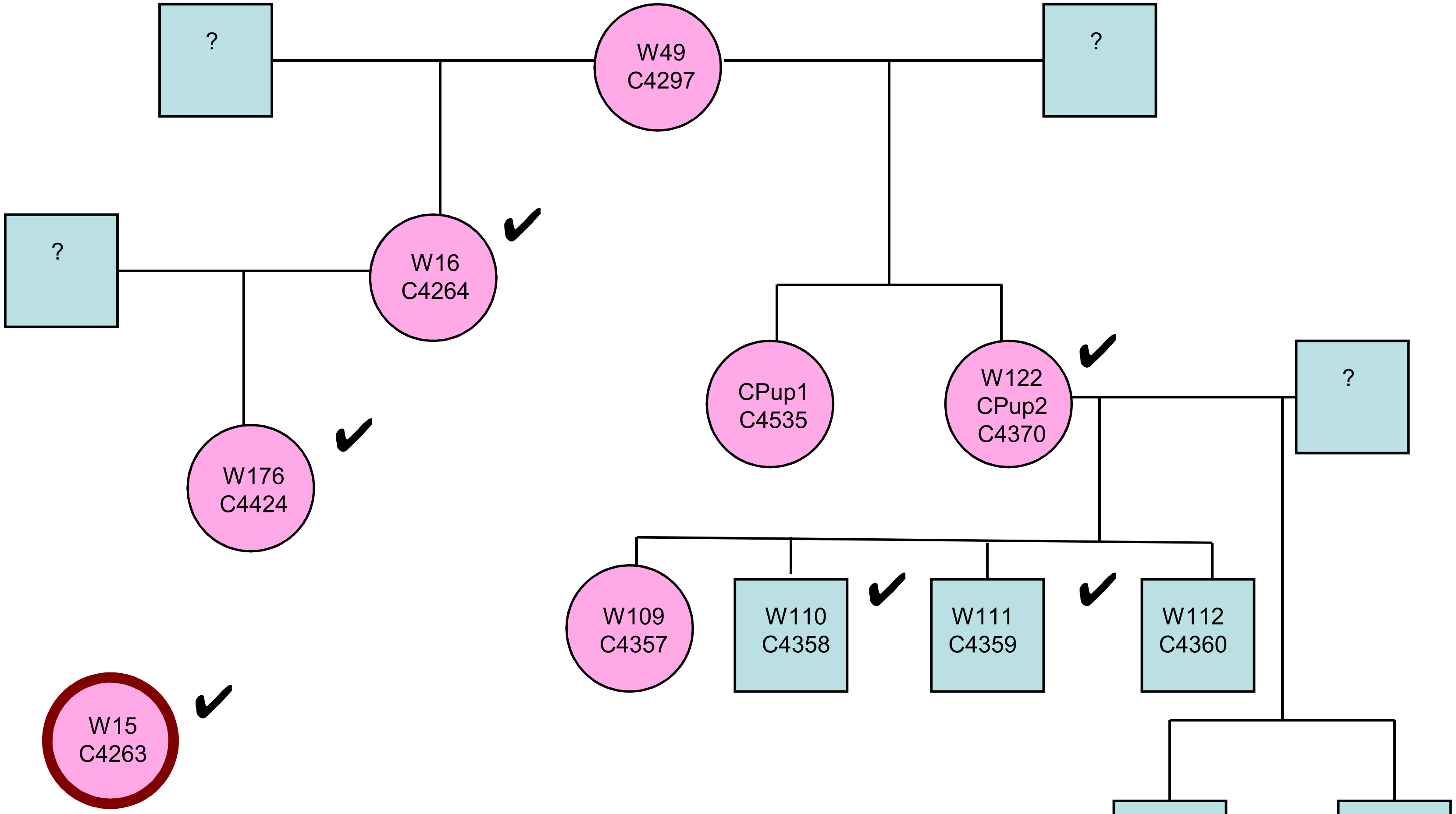
Big Crow



Supplementary Fig. 1d

Cauliflower

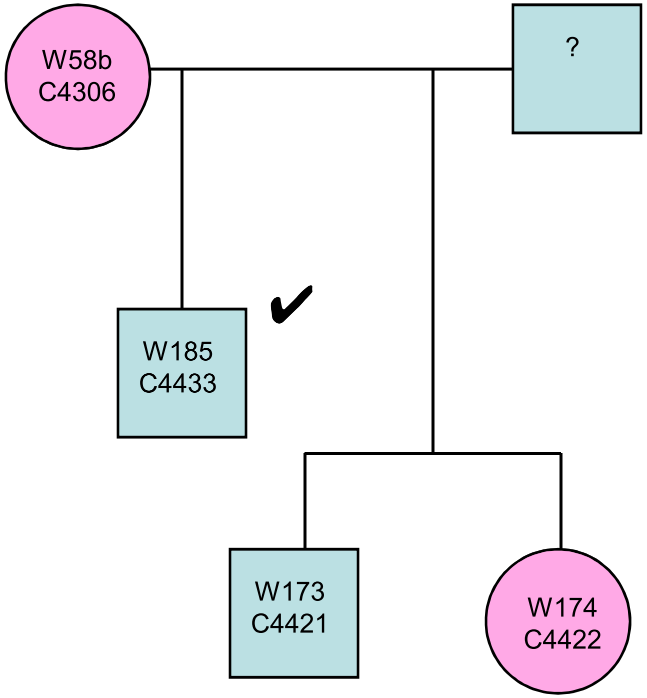
n=12



Supplementary Fig. 1e

n=4

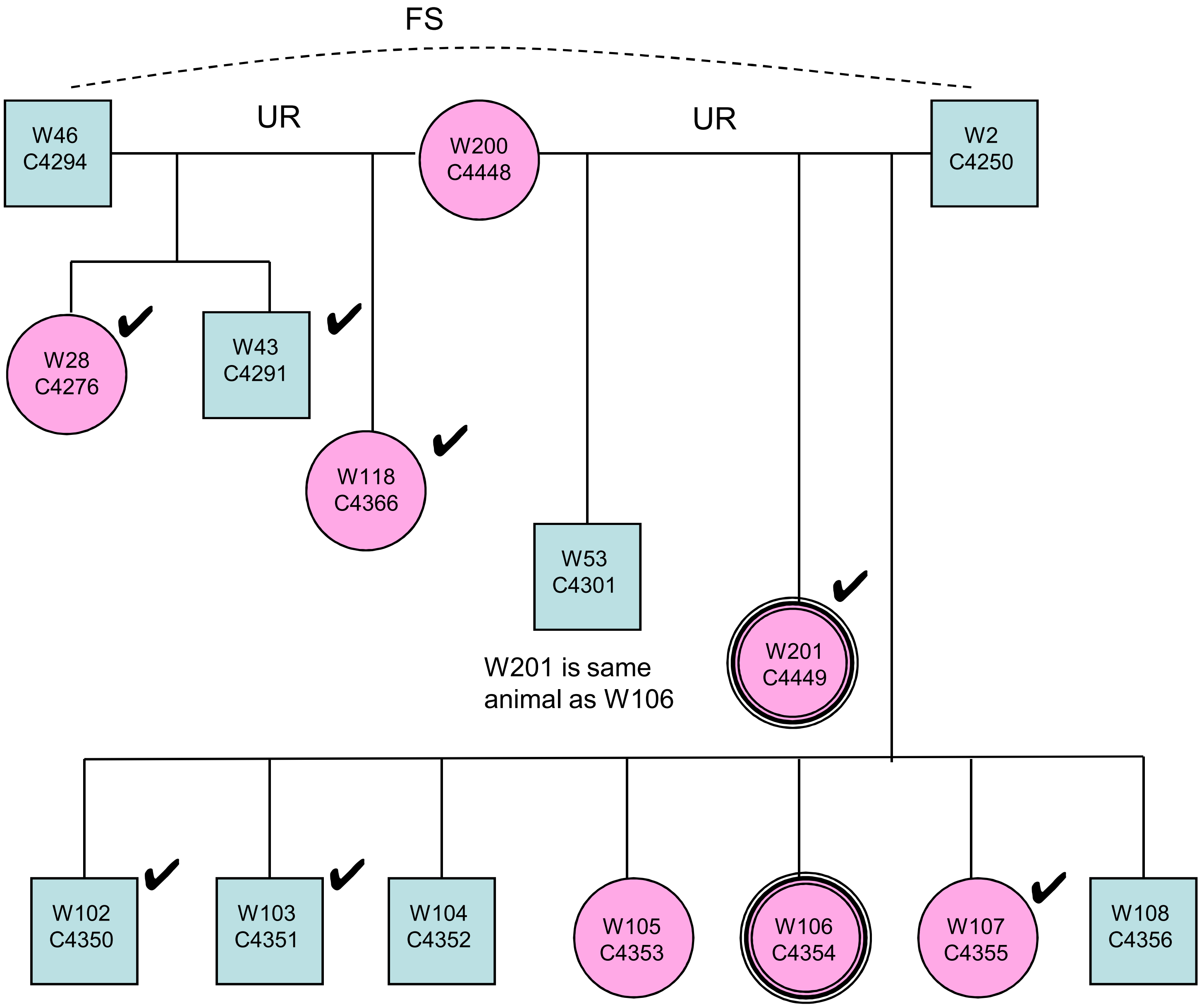
Flat Iron



Supplementary Fig. 1f

Jocko

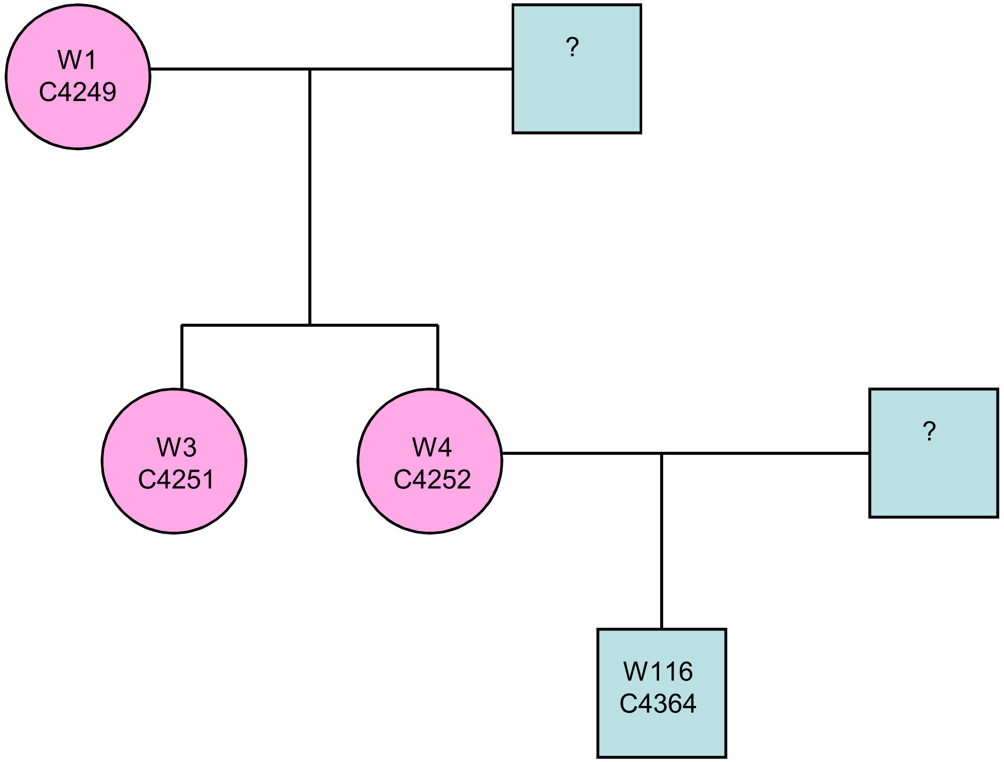
n=14



Supplementary Fig. 1g

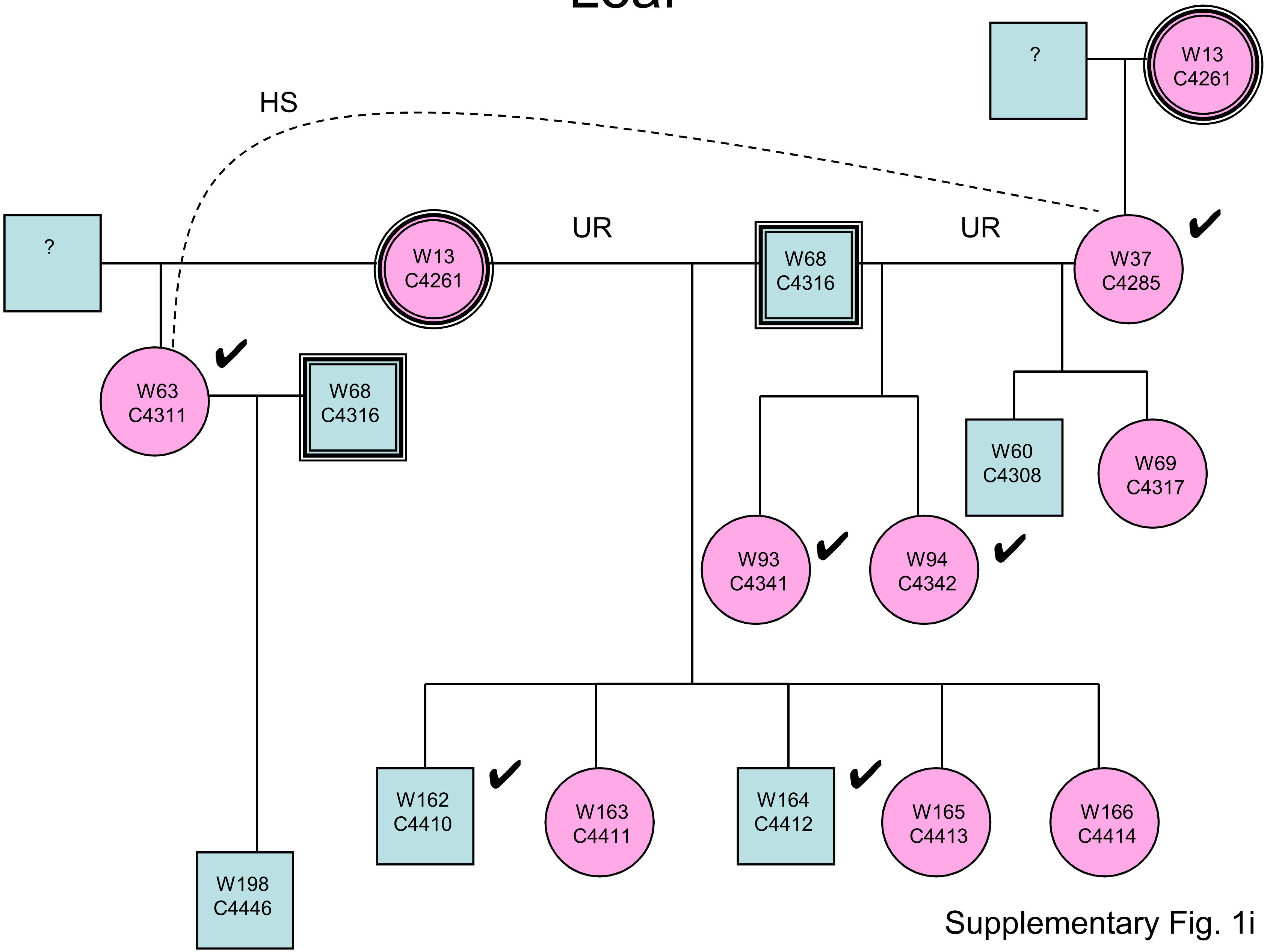
LaFleur

n=4



Supplementary Fig. 1h

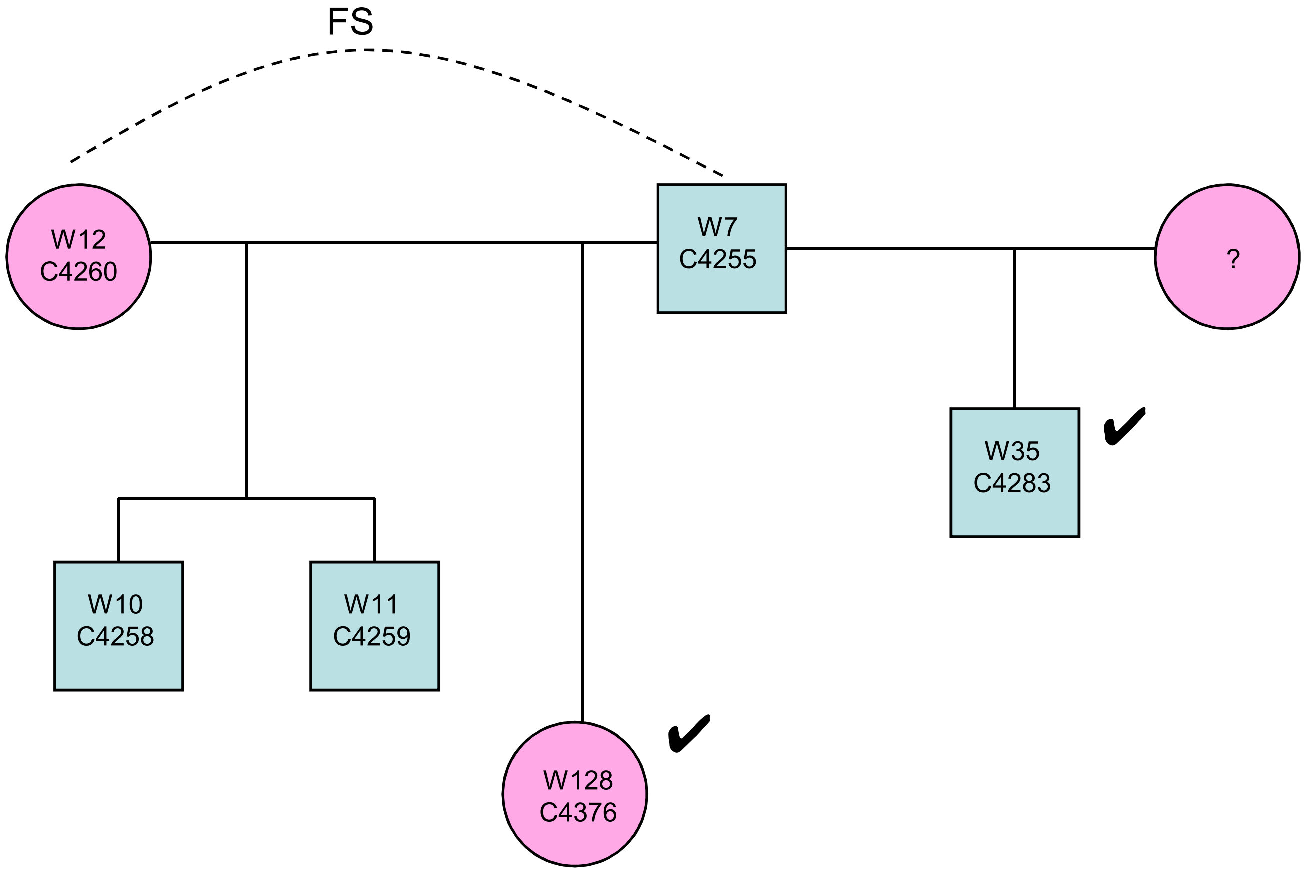
Leaf



Supplementary Fig. 1i

Louisa

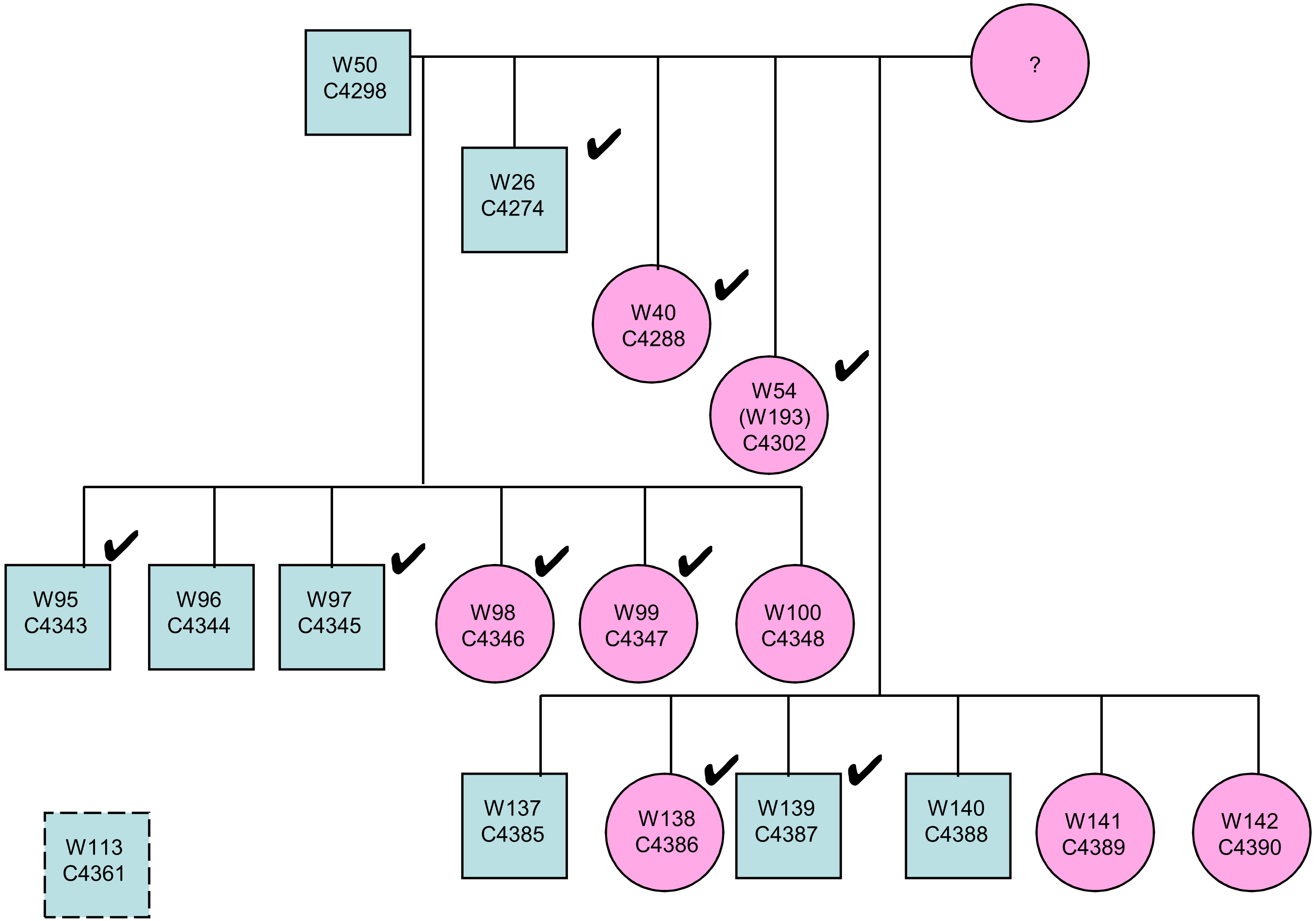
n=6



Supplementary Fig. 1j

McKaskill

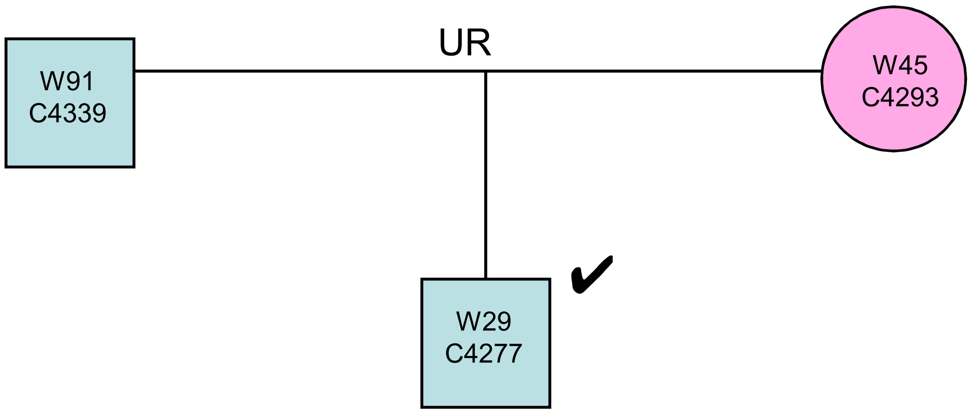
n=16



Supplementary Fig. 1k

Pine

n=3

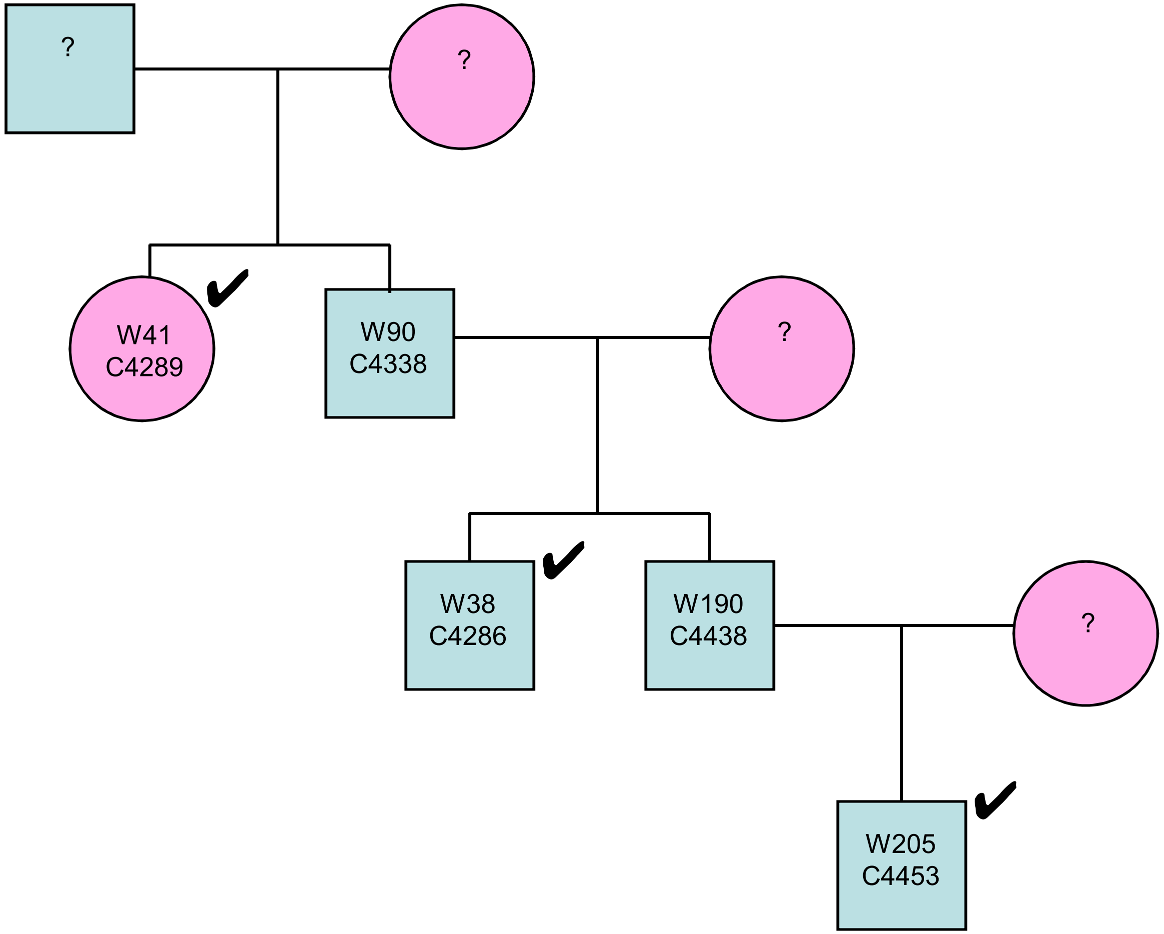


W45/C4293 was previously collared and tracked by John Theberge as a Zig Zag pack member during the pre-ban time period. She was recaptured and recollared during the post-ban study.

Supplementary Fig. 1l

Potter

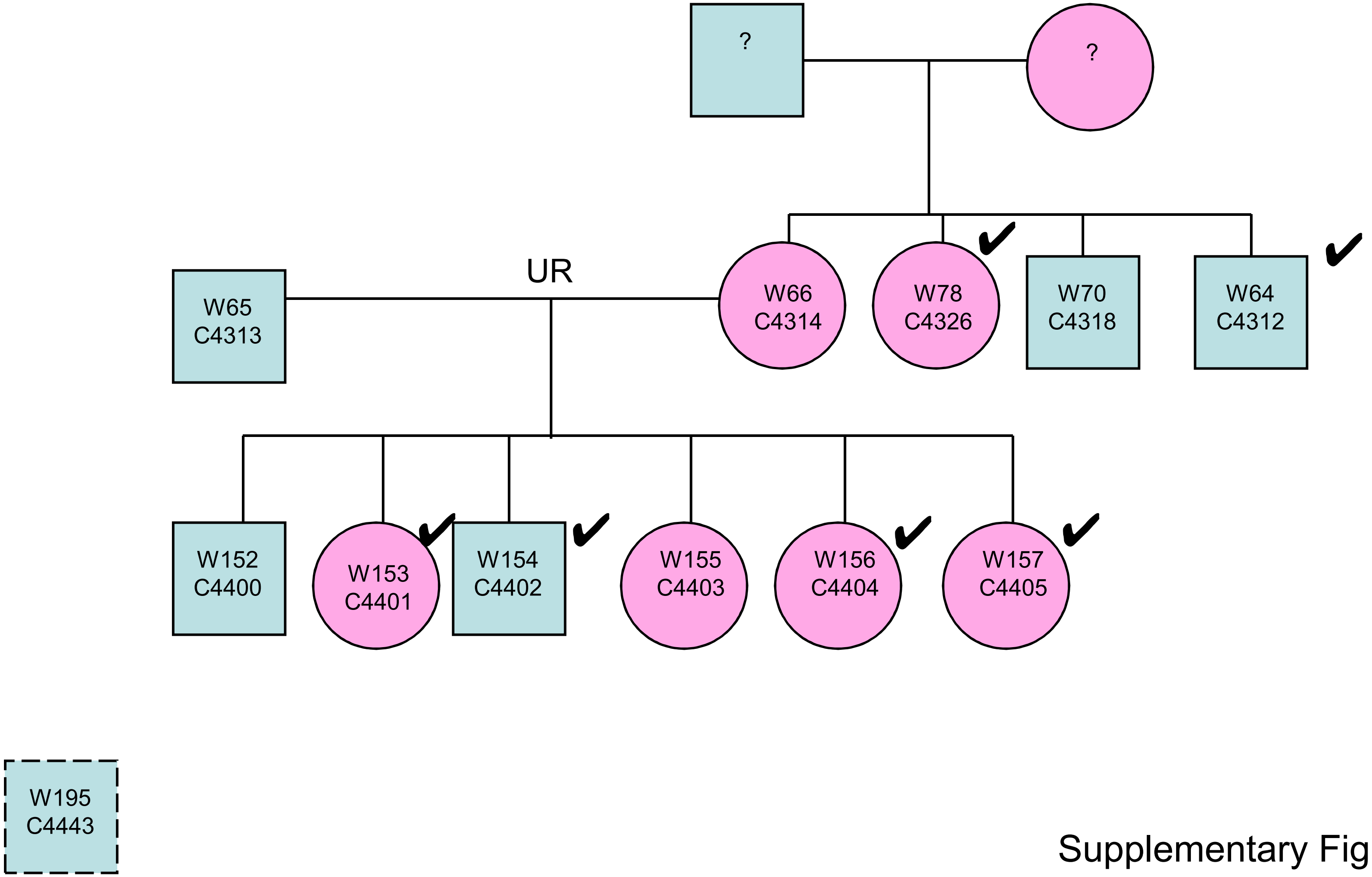
n=5



Supplementary Fig. 1m

Pretty

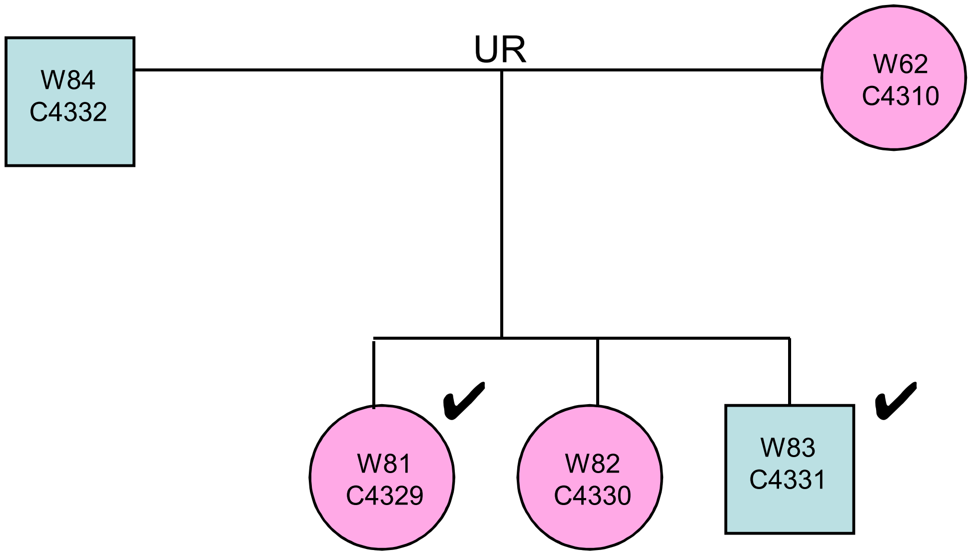
n=11



Supplementary Fig. 1n

Radiant

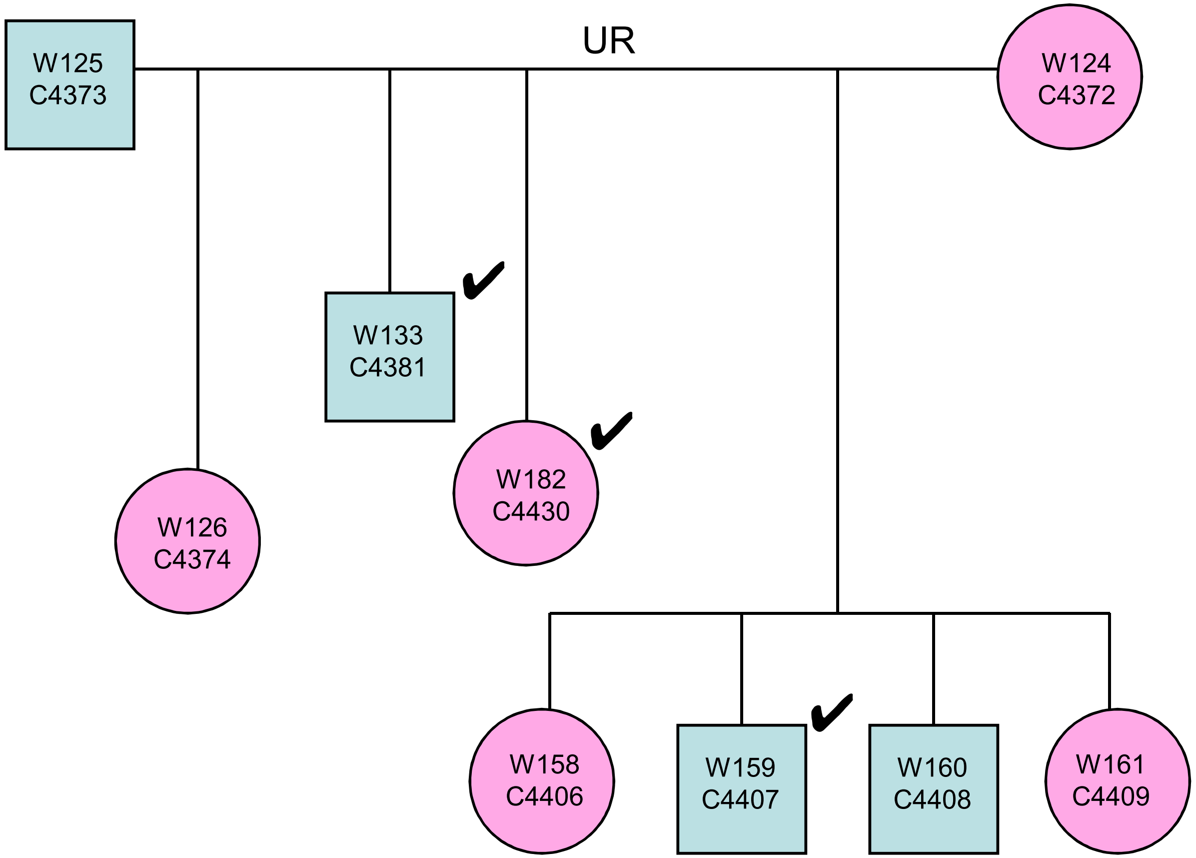
n=5



Supplementary Fig. 1o

Spoor

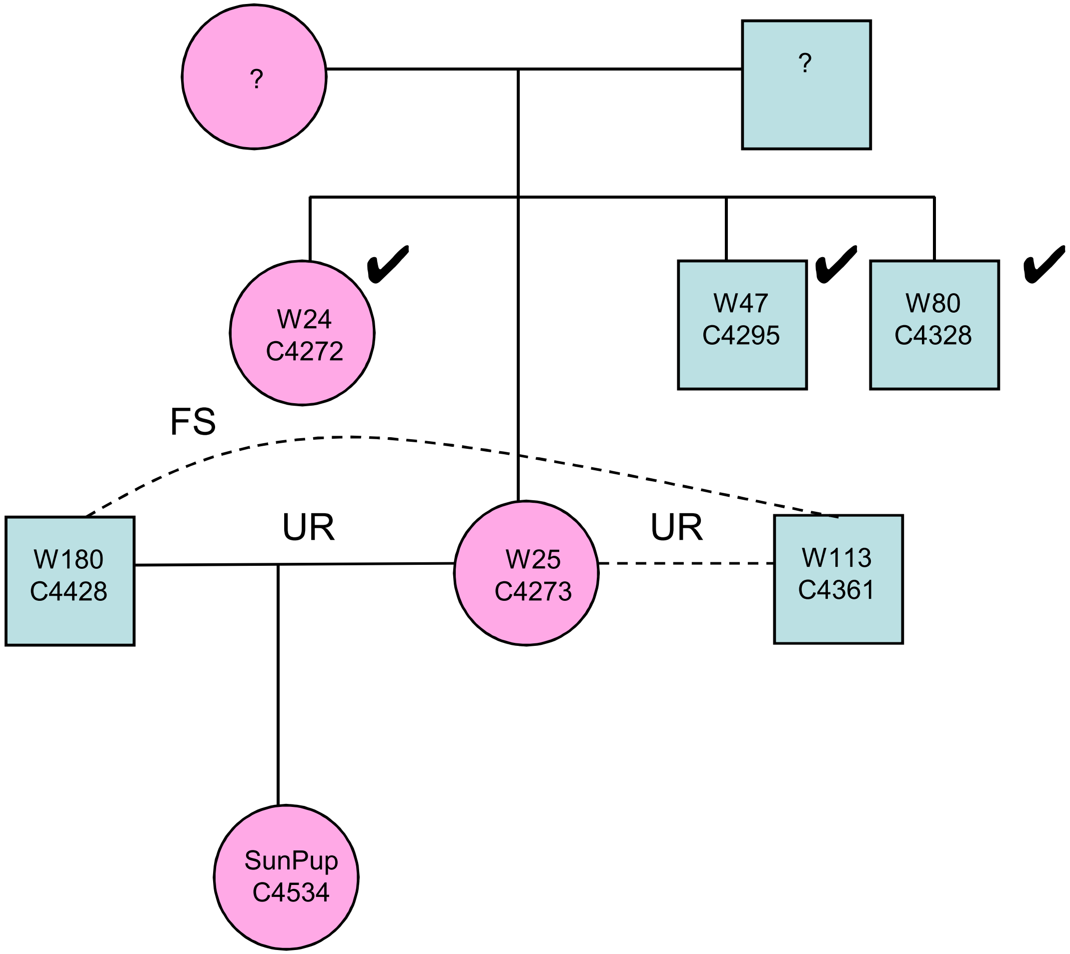
n=9



Supplementary Fig. 1p

Sunday

n=7



Supplementary Fig. 1q