

## Article

# Interface of Human/Wildlife Interactions: An Example of a Bold Coyote (*Canis latrans*) in Atlanta, GA, USA

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**Abstract:** There is arguably no other North American species that better illustrates the complexities of the human-wildlife interface than the coyote. In this study, a melanistic coyote in metropolitan Atlanta, Georgia was exhibiting unusually bold behaviors that included encounters with humans, domestic dogs, and attempts to enter homes. After tracking this coyote (nicknamed Carmine) across a highly urbanized landscape with participatory science, including at least 80 publicly reported sightings, he was captured and relocated to a wildlife sanctuary. Genome-wide analyses revealed 92.8% coyote ancestry, 1.7% gray wolf ancestry, and 5.5% domestic dog ancestry. The dog alleles in Carmine's genome were estimated to have been acquired by his ancestors 14–29 years ago. Despite his bold behavior, Carmine did not carry any mutations known to shape hypersociability in canines. He did, however, carry a single copy of the dominant mutation responsible for his melanistic coat color. This detailed study of Carmine dispels common assumptions about the reticent coyote personality and the origins of behavior. His unusual bold behavior created a higher level of human-coyote interaction. He now serves as a public ambassador for human-wildlife coexistence, urging the global community to reconsider mythologies about wildlife and promote coexistence with them in landscapes significantly altered by human activity in our rapidly changing world.

**Keywords:** coyote; urban wildlife; bold behavior; melanism; genotype; phenotype



**Citation:** Mowry, C.B.; Wilson, L.A.; vonHoldt, B.M. Interface of Human/Wildlife Interactions: An Example of a Bold Coyote (*Canis latrans*) in Atlanta, GA, USA. *Diversity* **2021**, *13*, 372. <https://doi.org/10.3390/d13080372>

Academic Editors: Joanna E. Lambert, Emily Beam, Amanda L. Ellwanger and Joel Berger

Received: 5 January 2021

Accepted: 5 August 2021

Published: 11 August 2021

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## 1. Introduction

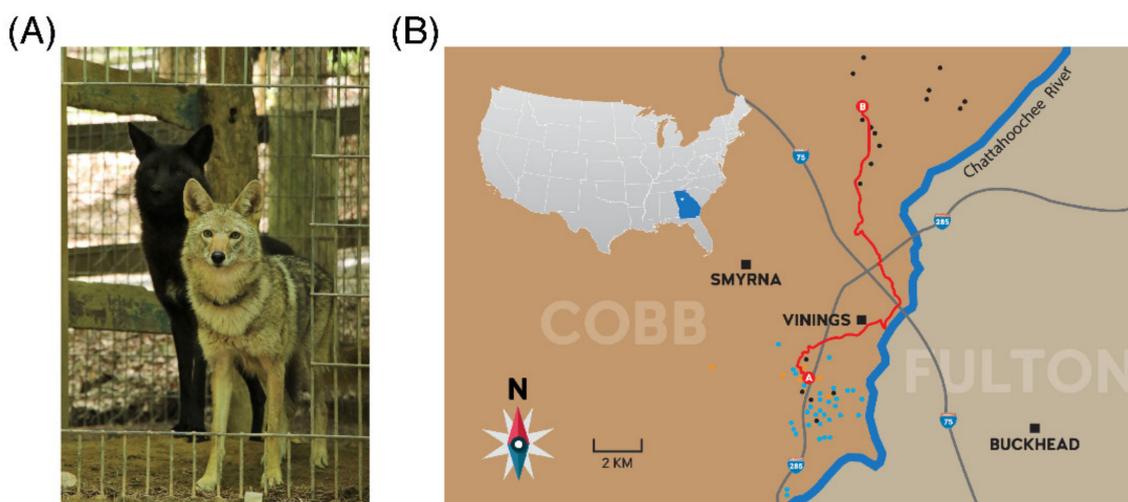
The evolutionary histories and successes of humans and dogs have been intertwined for more than 15,000 years ago, with details regarding the origins of dogs highlighted by ancient DNA studies [1]. The role of dogs in society has expanded dramatically since their origins, with a dramatic focus on identifying the genes linked to their behavioral and phenotypic evolution [1–11]. Simultaneously, society has remained challenged by coexistence with wild canids across the globe, and particularly with coyotes (*C. latrans*) for North American inhabitants. Both the domestication of wolves and more recent, eradication management efforts have shaped the physiology, morphology, genetics, and behavior of several canid species through introgression and admixture. This includes the North American coyote.

Several traits have evolved in dogs throughout the course of their domestication from gray wolves. Likely the most important evolutionary change is that dogs show an increased tolerance and interest toward humans relative to wolves [12]. Recent efforts discovered a few key genetic mutations that have contributed toward the hypersocial behavioral shift in canids [8]. These mutations segregate among wolf populations and dog breeds across the globe, but appear to be rare in coyote genomes. However, these mutations follow Mendelian inheritance and could thus be introgressed into other canid genomes via hybridization. Further, selective breeding of domestic dogs has altered their pigmentation types and patterning. Melanism is an additional trait that evolved in dogs

but was transferred back to wolves and coyotes through introgressive hybridization [13]. Although melanism is known to show an adaptive advantage maintained by balancing selection in gray wolf populations [14,15], coyote populations display a variable occurrence of melanistic and white coat colorations [16–19]. Although the evolutionary significance of the melanistic phenotype in coyotes remains yet to be documented, admixture and allele sharing among canids has been extensively documented [20–27].

The dynamic evolutionary history of the North American coyote has been dramatically shaped by human colonization, predatory control, and landscape conversion over the past few centuries. Historically found west of the Mississippi River, the coyote steadily expanded its geographic range during the past century and is now ubiquitous, living in nearly every major metropolitan area [28,29]. Human extirpation of congeneric red wolves (*C. rufus*) accelerated the southeastern range expansion of the coyote by eliminating its primary non-human competitor, while deforestation, urbanization, and the resulting increase in edge habitat were also all likely contributors [30–32]. In Georgia, coyotes first appeared in the central portion of the state in the 1960s and had a state-wide distribution just 20 years later [28,31,33]. Coyotes were prevalent in metropolitan Atlanta by the turn of the century and are now commonly observed throughout the region.

In this paper, we present genomic data derived from a bold coyote in Atlanta, Georgia. As we describe, this melanistic coyote lacked neophobia and exhibited proactive, exploratory, and unusually affiliative and friendly behaviors, including extensive solicitation of play from domesticated dogs [34,35]. The animal remained elusive to humans and was not overtly aggressive, nor did it attempt to copulate with dogs. Sightings of the animal, who was assumed to be a coyote and nicknamed Carmine (Figure 1A) by local residents, became commonplace over the next several weeks and its presence attracted widespread media attention due to public concern and curiosity. As a result, we initiated a plan to capture the animal in the interest of public safety, animal welfare, and scientific inquiry. Here, we provide data relevant to our goals of (1) understanding this animal's use of an urban landscape, (2) determining its ancestry and level of admixture with other *Canis* species, and (3) investigating if it carries any mutations associated with canine human-directed hypersocial behavior or melanism. Investigating patterns of admixture and novel genomic traits, including those related to melanism and hypersociality, can provide insight into the origins of inter-individual variation, speciation, and radiation of diversity.



**Figure 1.** (A) Carmine (melanistic coyote; left) and Wilee (agouti coyote; right) at the Yellow River Wildlife Sanctuary after Carmine's capture. (B) Map of publicly reported Carmine sightings in Cobb County, GA in December 2019 (yellow) and January (blue) and February (black) 2020. The red line represents the likely route taken by Carmine between 6 February (A) and 7 February (B) 2020.

## 2. Materials and Methods

### 2.1. Tracking and Capture of Subject Animal

We received daily public sightings of the study animal through the Atlanta Coyote Project's website (<http://cs.berry.edu/coyote/report.php>) and social media accounts (<https://www.facebook.com/atlantacoyoteproject>), which allowed us to track its movement, core areas of use, and distances traveled in the highly urbanized and fragmented landscape (Figure 1B). Concurrently, we learned that the Yellow River Wildlife Sanctuary (YRWS; Lilburn, GA, USA) was seeking a companion for a captive spayed female coyote (Wilee) that had been rehabbed but was unable to be released. With permission from the Georgia Department of Natural Resources, we targeted the study animal for capture and relocation to YRWS.

### 2.2. RAD Sequencing and Genomic Ancestry Analyses

We prepared genomic DNA from blood samples for restriction site-associated DNA sequencing (RADseq) following a modified protocol using the *SbfI* restriction enzyme and the NEBnext Ultra II DNA Library Prep Kit for paired-end ( $2 \times 150$  nt) sequencing on an Illumina NovaSeq6000 platform at Princeton University's Lewis Sigler Genomics Institute core facility [36]. We used STACKS v2 [37,38] to process and clean sequence reads for subsequent alignment to the reference dog genome CanFam3.1 assembly [39]. Ninety publicly available canid samples previously mapped to the same reference genome assembly were included as ancestry reference genomes (Table S1). We annotated SNPs across these 91 canid genomes and filtered them to retain sites with a MAF > 3%, with an allowance of up to 20% missingness per locus. For demographic analyses of genetic structure and diversity estimates, we constructed a "statistically unlinked" dataset of SNPs through linkage disequilibrium pruning. For full protocol and bioinformatic details, see Appendix A.

We assessed the probability of population membership to the reference groups using principal component analysis (PCA) and a Bayesian posterior probability assignment method (see Appendix A). Formal assignment of ancestry across the genome was completed with the program ELAI [40], which implements a two-layer hidden Markov model to estimate the most likely ancestry proportion at every locus. We implemented ELAI in serial triplicates to explore the parameter space and averaged the results. We estimated the timing that dog alleles first appeared in the study animal's ancestral genome through gene flow, and we followed the methods of Johnson et al. [41] to estimate the number of generations since admixture for diploid genomes (Appendix A). We utilized two estimates regarding the number of years in a canine generation (two and four years per generation) to capture possible events of canid yearling reproduction [42–47].

### 2.3. Genotyping Variants Associated with Canine Human-Directed Hypersocial Behavior

Due to the study animal's propensity to be extremely proximate to human activity, we hypothesized that it carried one or more genetic variants associated with increased human-directed sociability in dogs and wolves [8]. Although coyotes rarely carry these variants, if at all, this animal's behavior was exceedingly unusual. We used vonHoldt et al.'s [8] primers and PCR protocol to target four insertions sites for transposable elements (canine LINE and SINE transposons) on canine chromosome CFA6. Two insertions are associated with the gene *WBSCR17* (referred to as Cfa6.6 and Cfa6.7), one with the gene *GTF2I* (Cfa6.66), and one with gene *POM121* (Cfa6.83). Amplification and 2% agarose gel electrophoresis resolved the codominant status of copy number at each putative insertion site.

### 2.4. Sequence Assay for the Deletion Associated with K Locus Melanism in Canids

We genotyped the study animal for the 3bp indel at the gene *CBD103* (also known as the K locus) on canine chromosome CFA16:58,965,448 (canfam3.1 assembly) and followed the sequencing protocol of Cubaynes et al. (T. Coulson, personal communication). We used an amplicon-sequencing approach targeting the 3bp deletion. We mapped reads (122nt in

length) to the reference dog genome (CanFam3.1, GCF\_000002285.3) using *bwa-mem* [48], followed by indel genotyping with *GATK* v4.0.11.0 [49,50]. Further details are provided in Appendix A.

### 3. Results

#### 3.1. Behavior and Morphology

We received over 80 public sightings of the study animal between 24 December 2019 and 16 February 2020; sightings occurred at all hours of the day and night. On several occasions, Carmine was seen with at least one other coyote, although its consistent attempts to engage dogs (both male and female) in playful behavior always occurred in the absence of other coyotes (Videos S1 and S2). Based on reported sightings, the study animal spent seven weeks within a ~24 km<sup>2</sup> area of Smyrna and Vinings, GA, which meant it routinely crossed the I-285, one of metropolitan Atlanta's busiest interstate highways. We discovered three large culverts running under the I-285 within the study animal's core area, which likely offered the ability to avoid traffic. Two separate sightings occurred within the Smyrna, GA area on 6 February 2019, but 24 h later, the study animal was sighted and photographed 16.1 km away in the northeastern portion of Cobb County, where it utilized an ~11 km<sup>2</sup> area over the next ten days (Figure 1B). We captured the animal on 17 February 2020 after 49 days of trapping effort and determined that it was an approximately one-year old male that weighed 17.2 kg. Despite his bold behavior in the wild, he continued to actively avoid all human approach once in captivity and had to be sedated prior to physical examination. After surgical castration, he was gradually introduced to the resident coyote (Wilee) at Yellow River Wildlife Sanctuary and they immediately engaged in playful behavior when placed in the same enclosure.

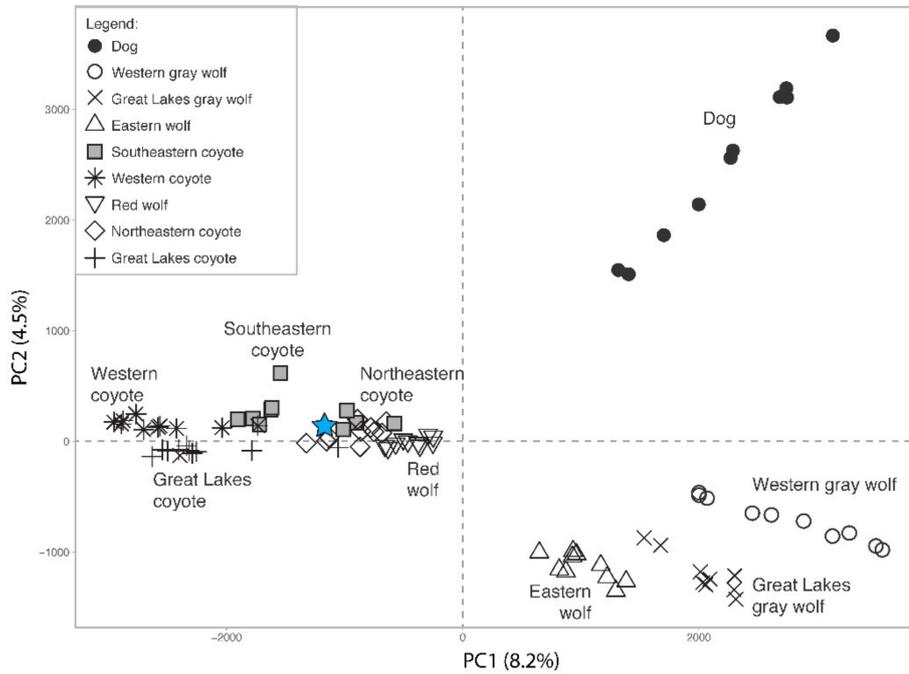
On 10 May 2020, four days after moving into his permanent exhibit with Wilee, Carmine escaped by scaling a 2 m-high enclosure fence and likely withstanding the electric shock that was delivered by the protective wire. He was monitored through a GPS collar showing that he immediately went to a nearby neighborhood and began playing with dogs, a route that had him swimming 30–35 m across the nearby Yellow River several times. Although he did not stray far, he remained at large for four days until his recapture.

#### 3.2. Ancestry

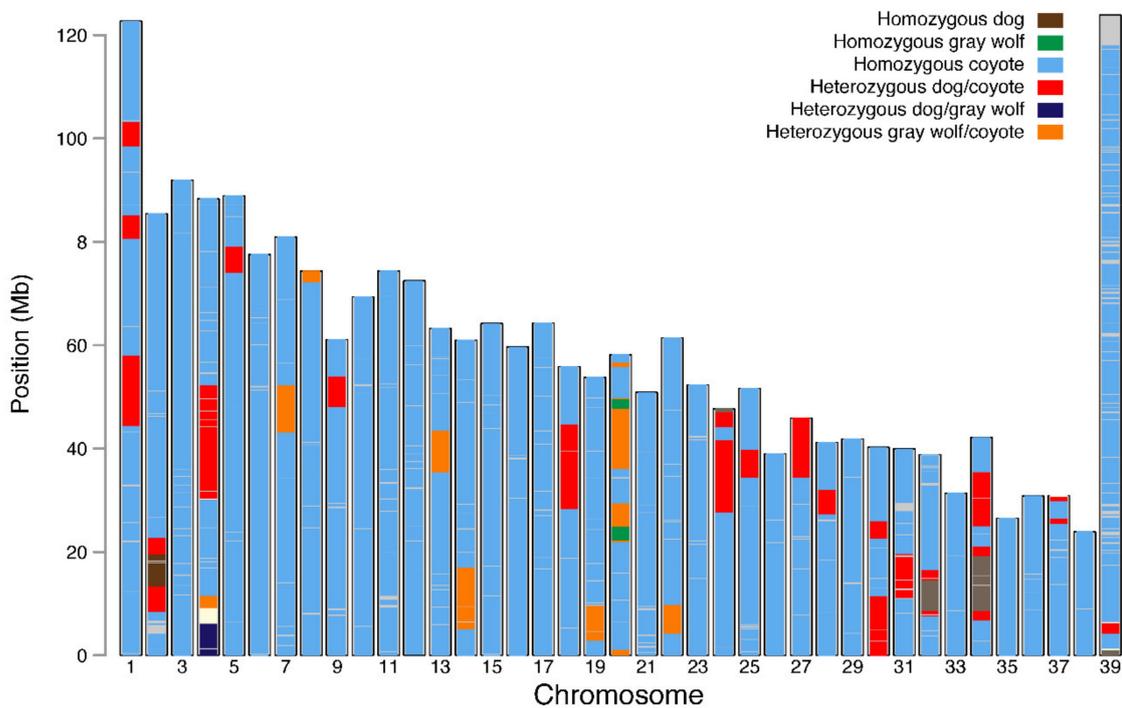
We assessed Carmine's genomic ancestry with respect to three reference species (domestic dog, gray wolf, and coyote) across 118,201 SNP genotypes (n, autosomes = 115,623; X chromosome = 2578) with an average marker density of one SNP variant every 19.7 Kb (density: autosomes = 19 Kb, X chromosome = 48 Kb). We estimated him to carry 92.8% coyote ancestry, 1.7% gray wolf ancestry, and 5.5% domestic dog ancestry (Figure 2; Tables S1 and S2). Across these loci, 103,069 sites were homozygous for coyote ancestry, 1330 were homozygous for dog ancestry, and 255 for gray wolf ancestry. Carmine carried 13,547 SNP loci that were heterozygous for ancestry states (n sites: dog/wolf = 366; dog/coyote = 10,110; wolf/coyote = 3071). For complete details of the RADseq SNP loci and assessment of reference genomes, see Appendix B.

It was possible to estimate the number of years ago when dog alleles first appeared in Carmine's ancestors' genome through gene flow. The total number of dog ancestry switches (autosomal = 30, X chromosome = 3) and genome-wide ancestry proportions specific to the type of chromosome (autosomes = 0.053, X chromosome = 0.124) were used for this estimation (Table S2). The dog alleles in Carmine's genome were estimated to have entered the ancestral genome 7.2 generations (14–29 years) ago, while the X-linked dog alleles were likely similarly acquired 6.2 generations (12–25 years) ago.

(A)



(B)



**Figure 2.** Genomic analysis of Carmine. (A) PCA of 105,931 SNP genotypes to cluster Carmine (star) with respect to 90 reference canids of North America. The percentage of variation explained by each component is provided in parentheses. (B) Chromosomal-level visualization of the ancestry across the 118,201 SNPs in Carmine’s genome. Ancestry was assigned to three reference canids of North America: domestic dogs, gray wolves (Western and Great Lakes), and coyotes (Western, Southeastern, Northeastern, and Great Lakes). The ancestry at each SNP was designated as either homozygous or heterozygous for the ancestry-informative allele. For detailed ancestry block information, see Table S2.

### 3.3. Canine Hypersociability and Melanism

Carmine lacked canine hypersocial transposons at all possible sites of integration. This is concordant with previously reported findings where such insertions are rare, at best, in coyote genomes [8]. We sequenced Carmine's *CBD103* gene to determine if his melanistic phenotype was due to the same 3bp deletion as previously reported to confer melanisms in gray wolves and some domestic dog breeds [13,51] and to determine its genotypic state. Carmine's *CBD103* gene was sequenced at an average depth of 506-fold coverage, and we determined him to be heterozygous for the deletion (coverage: wildtype allele = 232x, deletion = 236x; genotype quality (GQ) = 99).

## 4. Discussion

Carmine is a melanistic coyote who was exhibiting bold behavior in an urban environment at the interface of human-wildlife interactions. There is arguably no other endemic North American wildlife species that better illustrates the complexities of this interface than the coyote, and the story of Carmine's journey across metropolitan Atlanta is a perfect example of the circumstances that result in human-wildlife conflict versus coexistence. Carmine's ability to successfully navigate the matrix of the urban landscape prior to capture was particularly noteworthy as it demonstrated his keen awareness and understanding of his surroundings. Several recent studies have documented plasticity in coyote behavior in response to varying levels of urbanization [52–54], while Adducci et al. found genetic distinctiveness between urban and rural coyote populations [55]. Although urbanization has developed only relatively recently in the evolutionary history of canids, the short generation times and high fecundity rates of coyotes likely allow them to respond to such habitat selection pressures relatively quickly; this illustrates the role of diversity within the *Canis* genus over the past 1,000,000 years.

Carmine's bold behavior led to much public speculation about his genetic makeup, with many people believing that a coyote would never act in this manner unless it was the product of admixture with a dog, which was not the case. Although he lacks the hypersocial mutations associated with human-directed behavior in gray wolves and dogs, there are several other candidate genetic variants that likely contribute toward Carmine's bold personality type [56–58]. Previous work has quantified meaningful levels of additive heritability of the shyness-boldness spectrum of canine personality (~25% in dogs) [59]. Boldness has been linked to canine interest of playing with humans, interactions with strangers, and non-social fear [60]; such behavior is not novel for canines and has been quantitatively described in gray wolves [61]. Boldness is likely under environment-specific balancing selection where individuals who exhibit higher levels of boldness may experience benefits from resource acquisition (such as mates, food, territory). Mismatch to the environment, on the other hand, could result in deleterious outcomes (e.g., fatal interactions within urban landscapes). While urban coyotes have previously been found to be more bold and exploratory than coyotes living in rural habitats, there is little evidence that such behaviors are connected to aggression [62,63]. We are then faced with the question: can we attribute such personality to the efficiency at which eradication programs purge populations of bolder (less neophobic) individuals [62]? However, the effective population sizes of urban and rural coyotes are dramatically different, which impacts the efficacy of selection and the evolution of bold behaviors. Nonetheless, such behavioral differences serve as a reminder of the possible evolutionary options available when such standing phenotypic variation exists.

The decision to intervene in the life of a wild animal was not made lightly and it was not without some controversy. Typically, human-coyote interactions are rare and arguably the best management strategies are proactive measures that promote peaceful coexistence. Carmine's unusual bold behavior created a higher level of human-coyote interaction, but without governmental consent and the availability of a captive sanctuary, attempts to capture him would not have been undertaken. Nevertheless, he has now made significant

contributions to further our understanding of the interface of ecology and evolution while serving as a public ambassador for human-wildlife coexistence.

**Supplementary Materials:** The following are available online at <https://www.mdpi.com/article/10.3390/d13080372/s1>. Table S1: Sample information, population of origin, ancestry notes, proportion (prop.) of missing data per genome, and posterior probability (prob.) assignment to each possible reference genome. (Abbreviations: CFA, domestic dog; CLY, eastern wolf; CLU, gray wolf; CLA, coyote; CRU, red wolf). Bolded values indicate the new data collected for this study. Table S2: Posterior probability proportions (prop.) for each of the reference individuals with assignments up to two generations back. Notes are also provided to explain when admixture was detected among reference genomes and when samples were excluded from ancestry inference. (Abbreviations: gen, generation). Video S1: Carmine at the trap site. Video S2: Carmine playing with a dog.

**Author Contributions:** Conceptualization, C.B.M., L.A.W. and B.M.v.; methodology, C.B.M., L.A.W. and B.M.v.; formal analysis, C.B.M., L.A.W. and B.M.v.; investigation, C.B.M., L.A.W. and B.M.v.; resources, C.B.M., L.A.W. and B.M.v.; data curation, C.B.M. and B.M.v.; visualization, C.B.M., L.A.W. and B.M.v.; supervision, C.B.M., L.A.W. and B.M.v.; project administration, C.B.M.; funding acquisition, C.B.M. All authors contributed to writing and editing the manuscript. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research was funded by Berry College and through private donations.

**Institutional Review Board Statement:** The study was conducted according to the guidelines of the Declaration of Helsinki and the USDA Animal Welfare Act and Animal Welfare Regulations. It was approved under permits from the USDA (#57-C-0370) and USFWS (#MB36920D-0) on 31 December 2019. Trapping of the study animal was conducted under NWCO License #1000565487.

**Data Availability Statement:** The processed and mapped BAM file was deposited on NCBI's Short Read Archive (BioProject PRJNA682820). Additional data analyzed were previously published and details for their SRA information is found in references within Table S1.

**Acknowledgments:** We are grateful to Kris Hoffman, Brandon Sanders, Alex Taylor, Lara Shaw, Glenda Elliott, Vanessa Pryor, Jonathan Ordway, Katy Ordway, Abbey Elkert, Elizabeth Fincher, Clinton Murphy, Wayne Hubbard, Erin Brown, Bob Crabtree, Mark Johnson, and all others who contributed to the safe capture and care of Carmine. Craig Hall helped with figure preparation.

**Conflicts of Interest:** The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

## Appendix A

### Appendix A.1 Detailed Materials and Methods

#### Appendix A.1.1 RAD Sequencing and Bioinformatic Processing

High molecular-weight genomic DNA were obtained using the Qiagen DNeasy Blood and Tissue Kit. DNA was quantified by a Qubit 2.0 fluorometry system and we subsequently standardized the DNA sample to 5 ng/ $\mu$ L. Genomic DNA was prepared for restriction site-associated DNA sequencing (RADseq) following a modified protocol [36]. Genomic DNA was digested with *SbfI* and ligated a unique 8-bp barcoded with a biotinylated adapter, which allowed for subsequent pooling and demultiplexing after sequencing. Equal amounts of up to 48 samples were pooled followed by random shearing to 300–400 bp in a Covaris LE220, which was then enriched for adapter-ligated fragments using a Dynabeads M-280 streptavidin binding assay, followed by preparation for Illumina sequencing using the NEBnext Ultra II DNA Library Prep Kit. The library was then sequenced on an Illumina NovaSeq for paired-end ( $2 \times 150$  nt) reads at Princeton University's Lewis Sigler Genomics Institute core facility. Size selection and library purification were completed using Agencourt AMPure XP magnetic beads.

Sequence data were first processed to retain the reads (and their pairs) that contained the unique barcode and the remnant *sbfl* cut site. We used STACKS v2 [37,38] to demultiplex each sample using the *process\_radtags* module, with a 2bp mismatch for barcode rescue, and

retained reads with a quality score  $\geq 10$ . PCR duplicates were removed with the filtering option in *clone\_filter* with subsequent alignment to the reference dog genome CanFam3.1 assembly (GCF\_000002285.3) [39] using *STAMPY* v1.0.21 [64]. A mapping quality threshold was applied to retain mapped reads (MAPQ > 96) and convert them to the BAM format in *Samtools* v0.1.18 [48].

#### Appendix A.1.2 SNP Variant Discovery

SNP discovery was conducted using all 91 samples, ensuring that they each had a minimum of 100,000 mapped reads, to obtain a catalog of all polymorphic sites possible. The *gstacks* and *populations* modules in *STACKS* v2 were implemented as per default guidelines, with an increase of the significance threshold in *gstacks* and use of the Marukilow model (flags *-vt-alpha* and *-gt-alpha*,  $p = 0.01$ ) for variant discovery in the *populations* module. All SNPs discovered per locus were reported (we opted against using the *populations* flag *write\_single\_snp*) as ancestry inference is best with high-density data. Data were further filtered to retain sites with a MAF > 3% and to allow up to an 80% genotyping rate per locus (flag *-geno* 0.2) for the initial filtering step in *PLINK* v1.90b3i [65]. To determine if any individual sample was missing a significant proportion of genotypes, missingness was assessed using the *PLINK* function *-missing*, which excluded any samples with >50% missing data from all downstream analyses. For demographic analyses of genetic structure and diversity estimates, a “statistically unlinked” dataset of SNPs was constructed by excluding sites within 50-SNP windows that exceeded genotype correlations of 0.5 (with the *PLINK* argument *-indep-pairwise* 50 5 0.5). The X chromosome was analyzed independently from the autosomes to ensure the inclusion of the signal driven by the single sex chromosome captured in our sequencing and mapping efforts.

#### Appendix A.1.3 Population Structure Analysis

To survey genetic clustering and the structure of Carmine relative to the reference samples, two analyses were conducted with the statistically unlinked SNP set: principal component analysis (PCA) with the program *flashPCA* [66] and a Bayesian posterior-probability assignment test to assign Carmine to five possible genomic clusters ( $K$ ) in *STRUCTURE* v2.3.4 [67]. The parameter flags *MAPDISTANCES*, *POPFLAG*, and *USE-POPINFO* were used with the others at default settings for analysis at  $K = 5$  (i.e., dog, eastern wolf, gray wolf, red wolf, coyote) with 10,000 *BURNIN* and 20,000 *NUMREPS*.

#### Appendix A.1.4 Inference of Canid Ancestry

Carmine’s genomic ancestry was inferred with respect to three possible reference populations (gray wolves, coyotes, dogs) (Table S1). The full SNP set (unfiltered for statistical correlation) was analyzed with the program *ELAI* [40], which implemented a two-layer hidden Markov model to estimate the most likely ancestry proportion at every locus. Loci were excluded if they were lacking in any of the populations analyzed. The number of upper-layer clusters ( $-C$ ) was set to 3 (i.e., red wolves and each reference coyote population) and the lower-layer clusters ( $-c$ ) to 15 ( $5 \times$  the value of  $C$ , as recommended). To account for uncertainty in the precise timing and duration of admixture, four timepoints since admixture ( $-mg$ ) were analyzed: 5, 10, 15, and 20 generations ago. *ELAI* was implemented in serial triplicates for each parameter setting with 30 EM steps, with the results averaged over these replicates. Only sites with allele dosages >1.5 were considered to be homozygous and all other sites were heterozygous. Sites with multiple and incompatible ancestries (e.g., three heterozygous states) were excluded. An ancestry block was defined by a minimum of three contiguous SNPs with the same ancestry assignment.

The number of dog ancestry block switches was used to infer the timing at which dog alleles first appeared in Carmine’s ancestral genome through gene flow [41] by rearranging the equation for diploid genomes:

$$B = (2 \times 2 \times 0.01) \times T \times L \times z(1 - z)$$

where  $B$  is the estimated number of dog ancestry switches,  $T$  is the number of generations since admixture,  $L$  is the total genome length (2085cM for autosomes and 111cM for the X chromosome [68]), and  $z$  is the genome-wide ancestry proportion of dog ancestry across the autosomes or X chromosome.

#### Appendix A.1.5 Genotyping Variants Associated with Canine Human-Directed Hypersocial Behavior

Due to Carmine's propensity to be extremely proximate to human activity, it was hypothesized that perhaps he carried one or more genetic variants that are associated with increased human-directed sociability in dogs and wolves [8]. Although coyotes rarely carry these variants, if at all, Carmine's behavior was exceedingly unusual. The primers and protocol used were previously reported by vonHoldt et al. [8]. Briefly, PCR was used to target four insertion sites for transposable elements (canine LINE and SINE transposons) on canine chromosome 6. Two insertions are associated with the gene *WBSCR17* (referred to as Cfa6.6 and Cfa6.7), one with the gene *GTF2I* (Cfa6.66), and one with gene *POM121* (Cfa6.83). Amplification and 2% agarose gel electrophoresis resolved the codominant status of the copy number at each putative insertion site.

#### Appendix A.1.6 Sequence Assay for the Deletion Associated with K Locus Melanism in Canids

Carmine was genotyped for the 3bp indel at the gene *CBD103* on canine chromosome CFA16:58,965,448 (canfam3.1 assembly). This is known to be a deletion of the CCC fragment, often with the preceding nucleotide also deleted (TCCC indel). Briefly, a dual-barcoding index was constructed for short-read single-end sequencing of 122 nt on an Illumina NovaSeq6000 at Princeton University's Lewis Sigler Genomics Institute core facility. Adapter sequences were clipped (maximum error rate = 0.5, minimum overlap = 20) and a single mismatch for demultiplexing the pooled reads was allowed, which retained reads of 30nt or longer. Reads were mapped to the reference dog genome (CanFam3.1, GCF\_000002285.3) using *bwa-mem* (arXiv: 1303.3997v2). Read groups were edited using the *AddOrReplaceReadGroup* function in the Picard Toolkit (Broad Institute, 2019) and we sorted/indexed the BAM file with *samtools* v0.1.18 [48]. The *HaplotypeCaller* function of GATK v4.0.11.0 [69] was implemented to genotype the 3bp deletion using the flag -ERC GVCF. Only sites with at least 10-fold sequencing coverage were considered.

## Appendix B

### *RADseq Results*

From the RADseq method, 2,091,181 SNPs were discovered across 91 canid samples. After filtering for MAF and missingness, 146,206 SNPs were retained. On average, samples contained 11.4% missing genotypes (Table S1), and all samples were included for analysis. A second dataset was constructed by removing 4025 loci that displayed a high level of inter-locus correlation. This "statistically unlinked" dataset of 105,931 loci was used for genetic clustering and structure analyses. Both PCA and a posterior probability assignment test for the current generation correctly identified a known positive control, sample #2338, that was mislabeled as a Wisconsin gray wolf. This sample correctly and accurately clustered with Great Lakes coyotes ( $Q_{coyote} = 1.0$ ) (Figure 2; Table S2). This positive control sample was excluded from the ancestry inference. Additionally, sample #8682 (Kentucky coyote), had been assigned to 'dog' two generations back ( $Q_{dog} = 1.0$ ) and was also excluded from ancestry inference (Table S2). There were 11 additional samples (1 gray wolf and 10 coyotes) that were misclassified; however, their assignments were two generations back. All are consistent with previously published reports of admixture and shared demography. The gray wolf (Michigan) was assigned to eastern wolf [70]; eight northeastern coyotes were assigned to eastern wolf [70]; and two southeastern coyotes were assigned to red wolf [21,22]. These 11 samples were retained for ancestry inference due to their known

demographic history and admixture, and represent crucial genetic variation found within each of the canid lineages.

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