WILDLIFE DISEASE

Disease outbreaks select for mate choice and coat color in wolves

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We know much about pathogen evolution and the emergence of new disease strains, but less about host resistance and how it is signaled to other individuals and subsequently maintained. The cline in frequency of black-coated wolves (*Canis lupus*) across North America is hypothesized to result from a relationship with canine distemper virus (CDV) outbreaks. We tested this hypothesis using cross-sectional data from wolf populations across North America that vary in the prevalence of CDV and the allele that makes coats black, longitudinal data from Yellowstone National Park, and modeling. We found that the frequency of CDV outbreaks generates fluctuating selection that results in heterozygote advantage that in turn affects the frequency of the black allele, optimal mating behavior, and black wolf cline across the continent.

Variation in animal color is frequently used to assess the quality of potential mates and their fit to environmental conditions (*1*). In many species, color covaries with aspects of the environment such as latitude, weather, and the presence of specific parasites, food resources, and predators. An individual's color can signal its condition or immunological status (*2*). Honest signals need coloration to be correlated with fitness-associated traits, and under these conditions, this may select for particular mate choice strategies because individuals choose partners that maximize the expected fitness of their offspring (*3*). When the environment varies spatially, generating a cline in selection pressures, this could lead to landscape-level variation in coloration (*4*) and spatial variation in strategies of mate choice behavior (*5*).

Although rare at high latitudes, black wolves increase in frequency along a southwest cline toward forested areas in North America (*6*), with the highest frequencies at each latitude observed along the Rocky Mountains (Fig. 1A). The absence of geographical barriers that prevent gene flow, coupled with molecular signals of selection, points to regional variation in coat color being due to a cline in selection pressures (*7*).

Coat color in wolves (*Canis lupus*) is determined by genotype at the *K* locus gene *CBD103* (*8*). The ancestral wild-type *k* allele allows a normal *Agouti* and *Mc1r* gene interaction, resulting in gray coat color, whereas a three-nucleotide deletion in the *K* locus gene causes the protein to prevent *Agouti* function, leading to dominant inheritance of a black coat (*9*). After a single introgression event into a North American wolf population in the past 7250 years, the black allele has undergone a selective sweep, revealing one of the most rapid spreads of an adaptive variant known in vertebrates (*10*). The homozygote *KK* and the heterozygote *Kk* have indistinguishable black pelage but very different fitnesses (*11*). Therefore, coat color phenotype is not itself under direct selection, but the black allele must have a function that affects fitness directly or through pleiotropic effects, conferring a strong selective advantage in certain environments (*7*, *12*). Because the *K* locus encodes for a β-defensin protein that plays a direct role in innate and adaptive immunity in mammals (*13*), we postulate that it is involved in immunity to respiratory infections such as the morbillivirus that causes canine distemper virus (CDV), a pathogen of carnivores (*14*) that can cause substantial mortality among immunologically naive individuals, particularly juveniles (*15*).

The ability to fight disease can generate a fitness cost in the absence of these threats (*16*, *17*). We investigated whether the environment-dependent fitness benefits of certain genotypes could explain the North American cline in wolf coat color frequency. CDV infects most carnivores, and the frequency of outbreaks varies depending on the composition of the carnivore communities (*18*). To test the prediction that coat color varies with CDV occurrence, we analyzed 12 wolf populations to determine whether the probability of a wolf being black was predicted by the presence of CDV antibodies (*19*).

Wolves seropositive for CDV are more likely to be black, especially at older ages (Fig. 1B). We constructed a model to assess the individual- and population-level effects of CDV on the probability of a wolf being black (*19*). We predicted the probability of being seropositive for CDV while standardizing for age and confounding factors (fig. S2). The population effect is the positive correlation between the population-level disease exposure and whether an individual is black or gray (Fig. 1C). The individual effect captures whether a previously CDV-exposed individual is more likely to be black, perhaps because it is more likely to survive the infection and be sampled later. We found that if an individual was seropositive for CDV, then its probability of being black increased from 25 to 32% (P = 0.03) (fig. S4). These results are consistent with our hypothesis that CDV exposure is positively associated with coat color frequency and provide extensive comparative support.

Next, we report an analysis of the Yellowstone wolf population using individual life history and coat color data collected since their reintroduction in 1995–1997 (*19*). The population consists of ~55% gray wolves (genotype *kk*) and 45% black wolves (genotypes *Kk* and *KK*), with only 5% of these being homozygotes (Fig. 2). Previous research has revealed that female gray wolves have 25% higher annual reproductive success in all years compared with black females, and that CDV outbreaks generate a 50% reduction in female reproductive success independently of coat color (*20*). However, because so few genotyped black homozygote individuals have reproduced in Yellowstone (n = 5), there is insufficient statistical power to determine whether there is any difference in reproductive performance between black genotypes. A survival advantage of black heterozygotes over the other two genotypes across all years has previously been reported (*11*), but we do not know whether coat color and CDV infection interact to influence survival, and therefore the relative fitness, of the three genotypes.

We used longitudinal data to explore how annual age-specific survival rates varied between 1998 and 2020 among homozygote black, heterozygote black, and homozygote gray wolves with individual exposure to CDV during five CDV outbreaks (*19*). We developed a mark-recapture model that included transitions among susceptible, exposed, and immune states. We also included information on permanent dispersal and non-natural and known natural deaths while simultaneously modeling recapture rates. Pack identity and year were included as random effects.

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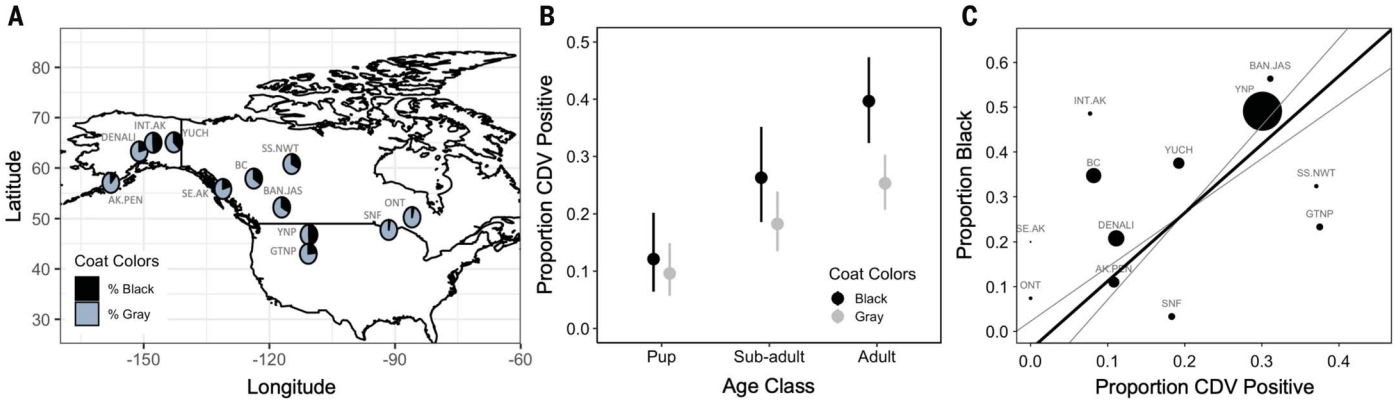

Fig. 1. Occurrence of CDV and coat color in wolves across North America. (**A**) Proportion of each coat color phenotype. Wolf sampling locations included Alaska Peninsula (AK.PEN), Denali National Park (DENALI), interior Alaska (INT.AK), Yukon Charley Rivers National Preserve (YUCH), southeastern Alaska (SE.AK), British Columbia (BC), South Slave Northwest Territories (SS.NWT), Banff and Jasper National Parks (BAN.JAS), Yellowstone National Park (YNP), Grand Teton National Park (GTNP), Ontario (ONT), and Superior National Forest (SNF). YNP and GTNP are offset for visual purposes (n = 1274) (*19*). (**B**) Proportion of pup, subadult, and adult wolves seropositive for CDV among N = 1134 with known age and sex from the wolf populations sampled in (A). Also shown are 95% confidence intervals (95% CIs). (**C**) Relationship between CDV prevalence and proportion of wolves with black coat color. The thick black line is a restricted major axis regression weighted by sample size, and gray lines show the 95% bounds of the regression estimate. Circles are scaled to sample size (N = 1166).

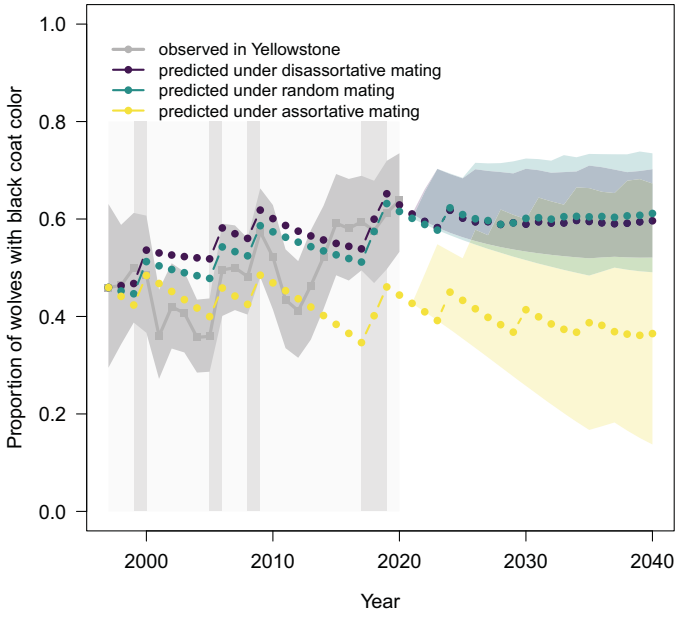

Fig. 2. Observations and predictions of the frequency of black wolves in Yellowstone National Park. Dark gray lines are point estimates. Shaded area represents 95% CIs. Vertical gray lines represent past CDV outbreaks. Up to 2020, colored lines and dots represent a single model projection accounting for past CDV outbreaks for random (green), disassortative (purple), and assortative (yellow) mating. After 2020, the colored shaded areas represent 95% CIs estimated from 500 model runs assuming the same frequency of CDV outbreaks (annual probability of 0.2).

Mirroring the results of our broad-scale surveys, our analyses revealed that black heterozygote wolves have higher survival compared with gray wolves, but only in CDV-infected individuals (Fig. 3). Because inheritance at the K locus is Mendelian, if the survival advantage to the heterozygote exposed to CDV compensates for the reduced fertility of black females, then it may be advantageous to mate with a partner of the opposite color to maximize the likelihood of producing heterozygote offspring when epizootics are frequent. We thus hypothesize that fluctuating, frequency-dependent selection due to CDV outbreak frequency can alter the relative fitness of the genotypes, resulting in a fitness advantage to heterozygotes when disease is frequent enough and selecting for the disassortative mating strategy observed in Yellowstone (*21*).

We constructed a stochastic, demographic, two-sex model of the dynamics of the three genotypes (*19*). We used a mating function that allowed us to alter the mating preference from random through disassortative to assortative and evaluate which mate choice strategy was optimal under various disease outbreak frequencies.

When the model was parameterized with initial starting conditions equal to the observed coat color frequencies at reintroduction into Yellowstone and observed CDV outbreaks, the simulations captured the observed dynamics of coat color frequency adequately when we assumed random or disassortative mating (Fig. 2). The model did not perform well with assortative mating, consistent with the excess of black-gray pairs reported in Yellowstone (*21*). Therefore, despite its simplicity, our model captures the dynamics of wolf coat color genotypes in Yellowstone.

Our model predicted that the frequency of black wolves depends upon the frequency of CDV outbreaks and mate choice strategy (Fig. 4A). Under all mate choice strategies, the frequency of black wolves increased with the frequency of CDV outbreaks. The rate of increase was steepest when wolves mated assortatively and shallowest when they mated disassortatively. In Yellowstone, wolves mate disassortatively, but is this adaptive?

Disease-induced mortality selects for the evolution of mate choice, but the evolutionarily stable strategy (ESS) changes on either side of a threshold in disease frequency (*19*). Below an outbreak frequency of 0.1 (≈1 outbreak every 10 years), an assortative mating strategy is the ESS (Fig. 4B), whereas above it, a disassortative mating strategy has greater fitness. Random mating is never the ESS. The black allele is always eliminated in the absence of CDV when the ESS is assortative mating, whereas disassortative mating results in a stable polymorphism.

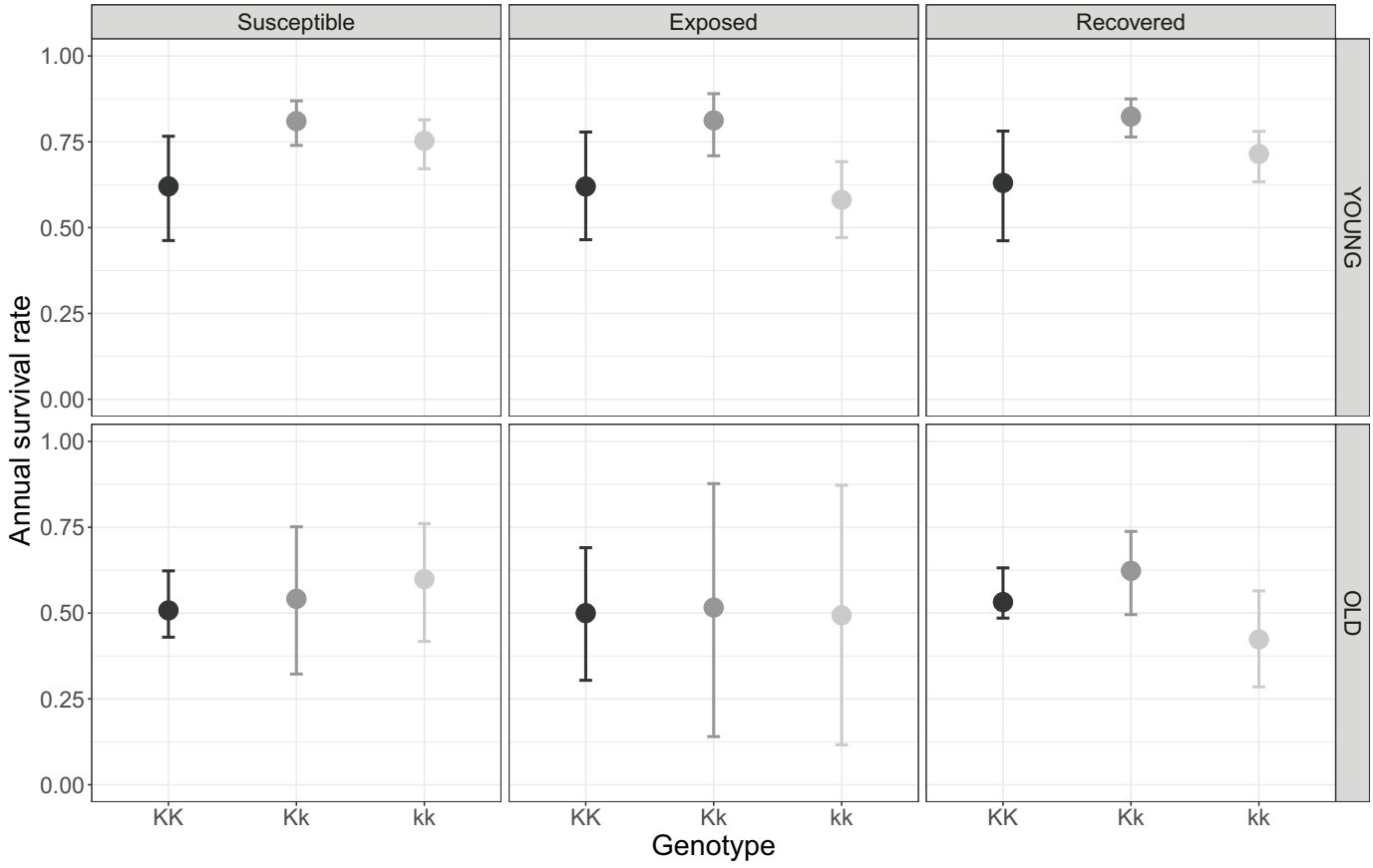

Fig. 3. Survival analysis results. Effects of age (top, young; bottom, old), disease status (columns), and *K* locus genotype (KK for homozygote black, Kk for heterozygote black, and kk for homozygote gray) on survival rates. Medians with 80% credible intervals are displayed.

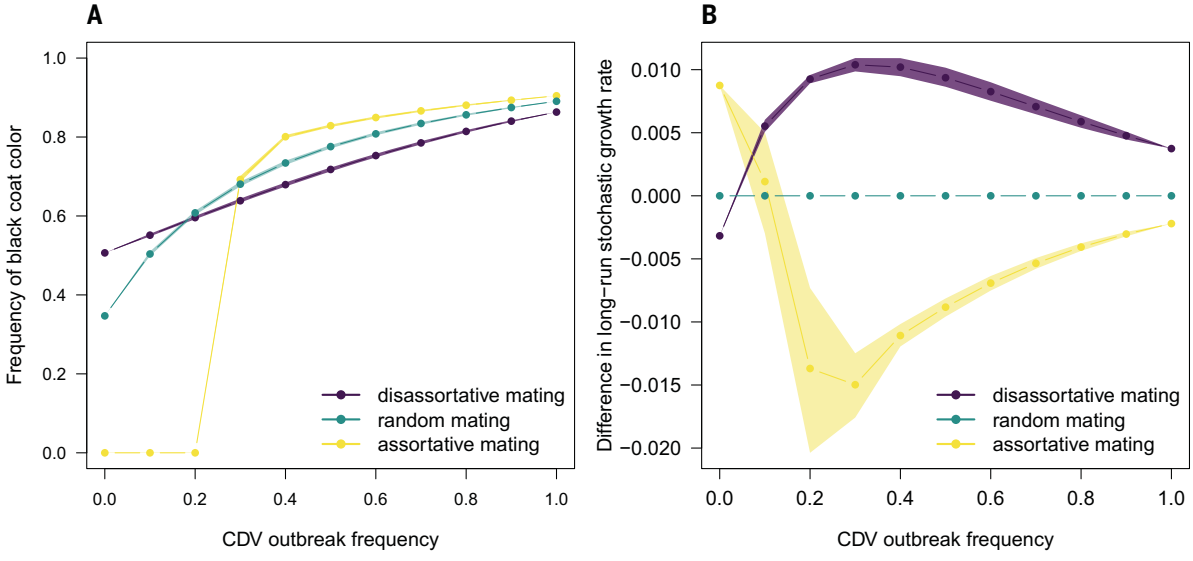

Fig. 4. Model predictions as CDV outbreak frequency varies. (**A**) Effect of CDV outbreak frequency and mating system on the frequency of black wolves. (**B**) Difference in strategy fitness relative to random mating (green) for assortative (yellow) and disassortative (purple) mating strategies as a function of CDV outbreak frequency. Lines represent point estimates, and shaded polygons represent 95% CIs from 500 simulations.

Our modeling results are consistent with our hypothesis that the frequency of disease outbreaks is responsible for the observed cline in coat color seen across North America, and they also explain why Yellowstone wolves mate disassortatively. We would expect an assortative mating strategy when CDV outbreaks occur less than once every decade. Although our results are consistent with observations, recent laboratory experiments challenging wolf cell cultures with a range of pathogens have so far failed to discern genotype-specific responses to CDV (*22*). Although elegant, that work cannot answer the question that we have addressed because it fails to capture susceptibility to infection

and the complexity of immune responses expected within free-living individuals (*22*). In addition, genetic findings have reported positive selection on coat color genes, major histocompatibility complex (MHC) genes, and immunity genes along a gradient of temperature and humidity (*7*), a finding that is consistent with our conclusions.

Our results are unlikely to be specific to wolves. In many insects, amphibians, reptiles, birds, and nonhuman mammals, disease resistance is associated with coloration (*1*, *2*, *12*, *23*), a trait that can act as a signal for pathogen resistance in mate choice (*2*, *3*). Recent findings have identified associations between disease-resistance MHC genes and coloration in mammals (*10*, *24*), amphibians (*25*), reptiles (*26*), and birds (*27*), possibly through pleiotropic effects or the action of "supergenes" (*1*, *28*). For example, in some bird species, carotenoid-dependent coloration (*23*) can drive mate choice through associations with disease-resistant MHC genes that influence the sensory functions of odor, vision, and hearing (*24*).

When coloration is genetically determined and disease resistance is heritable and associated with coloration, a preference for a mate of a specific color will enhance fitness by maximizing the chances of producing resistant offspring in environments with frequent and virulent enough pathogens. When the environment varies spatiotemporally, alternative mating strategies could explain the maintenance of color polymorphism (*5*) through negative-frequency–dependent selection, as shown here for wolves. Incidental color biproducts of immune response genes may consequently be widespread drivers of the mating behaviors observed across a diverse array of animal species. It is possible that we have significantly underestimated the role of pathogens in generating the diversify of morphological and behavioral traits observed in nature (*25*, *29*).

CDV requires a high population density to persist, and because wolves live at low densities, CDV cannot be endemic within a population (*18*). Instead, it requires a broad community of carnivores to persist and intermittent spillover transmission back to wolves. The reservoir community, species, or population is not well understood for CDV in North America, but we show that CDV prevalence is positively associated with human density (fig. S4) (*19*). CDV probably evolved from human measles epidemics that decimated indigenous South American populations when the virus spilled over into the abundant dog population and evolved into CDV before being found in North America in the 1760s (*30*). Wolves have genes for black coat color because they reproduced with the dogs of First Nations People, and this introgression happened between 1598 and 7248 years ago (*10*). It is therefore likely that other pathogens or mechanisms have contributed to the rapid spread of the black allele.

None of our analyses, on its own, provides conclusive support for the hypothesis that the frequency of black wolves across North America is determined by the frequency of CDV outbreaks, but each separate, complementary line of evidence provides support. These results are important because, first, they reveal how the frequency of disease outbreaks imposes selection on immune function, generating heterozygote advantage only under certain environments, similar to what is seen with sickle cell disease in humans. In the absence of CDV in the environment, the dominant *K* allele in wolves is expected to be lost because an assortative mating strategy would be selected, with the frequency of CDV outbreaks determining the frequency of the derived *k* allele. We provide support that variation in CDV outbreak frequency has generated the cline in wolf coat color observed across North America. An incidental biproduct of genetic variation at the *K* locus is coat color variation, a marked phenotypic pattern that has long puzzled researchers. The second line of evidence is that sexual selection has operated on this incidental cue to sculpt wolf behavior, with Yellowstone wolves mating disassortatively to maximize their fitness. This shows not only how the effects of a pathogen influence selection for resistance, but also how this is signaled between hosts and thus the mate choice behavior mechanism that results in host diversity. Finally, the results of our study show the true value of coupling geographically restricted, intensive, long-term, individual-based studies of wild populations with continent-wide, cross-sectional samples from multiple populations. We were able to link statistical results across these disparate forms of data using evolutionarily explicit population modeling. In doing so, we learned that the maintenance of genetic, morphological, and behavioral variation both within and between populations of a charismatic carnivore is the result of environmentally determined, fluctuating, frequency-dependent selection.

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Data and materials availability: Data and code used in this paper can be downloaded from Dryad (*31*) and Zenodo (*32*), respectively.

SUPPLEMENTARY MATERIALS

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Materials and Methods
Figs. S1 to S12
Tables S1 to S3
References (*33*–*60*)
MDAR Reproducibility Checklist

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