ASSESSMENT OF VARIATION IN THE DETECTION AND PREVALENCE OF BLOOD PARASITES AMONG SYMPATRICALLY BREEDING GEESE IN WESTERN ALASKA, USA

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ABSTRACT: Haemosporidian parasites may impact avian health and are subject to shifts in distribution and abundance with changing ecologic conditions. Therefore, understanding variation in parasite prevalence is important for evaluating biologically meaningful changes in infection patterns and associated population level impacts. Previous research in western Alaska, US, indicated a possible increase in *Leucocytozoon* spp. infection between Emperor Geese (*Anser canagicus*) sampled in 1996 (<1%, n=134) and during 2011–12 (19.9%, 95% confidence interval [CI]: 3.0–36.8%, n=77); however, different detection methods were used for these estimates. Prior research in this same region identified a lack of *Leucocytozoon* spp. parasites (0%, n=117) in sympatrically breeding Cackling Geese (*Branta hutchinsii minima*) in 2011. We molecularly screened blood samples collected from sympatrically breeding Emperor and Cackling Geese in western Alaska during additional breeding seasons to better assess temporal and species-specific variation in the prevalence of blood parasites. We found similar prevalence estimates for *Leucocytozoon* spp. parasites in Emperor Goose blood samples collected in 1998 and 2014, suggesting consistent infection of Emperor Geese with blood parasites at these time points. Using samples from sympatric geese sampled during 2014, we found evidence for a higher incidence of parasites among Emperor Geese (20.3%, 95% CI: 11.8–32.7%) compared to Cackling Geese (3.6%, 95% CI: 1.1–11.0%), reinforcing the previous finding of species-specific differences in infection. Furthermore, we detected *Leucocytozoon*, *Haemoproteus*, and *Plasmodium* spp. blood parasites in unflighted goslings of both species, supporting the possible transmission of these parasites at western Alaska breeding grounds. Our results help to clarify that prevalence of *Leucocytozoon* spp. parasites have probably remained consistent among Emperor Geese breeding in western Alaska since the late 1990s and that this species may disproportionally harbor *Leucocytozoon* spp. compared to sympatrically breeding Cackling Geese.

Key words: Avian haematozoa, blood parasite, Cackling Goose, Emperor Goose, haemosporidian, *Leucocytozoon*.

INTRODUCTION

Haemosporidians are a diverse group of single-celled, intracellular parasites that affect a wide range of vertebrates (Atkinson and van Riper 1991). Avian hosts are commonly affected by haemosporidians of the genera *Leucocytozoon*, *Haemoproteus*, and *Plasmodium*. These parasites are transmitted by dipteran insect vectors, with *Leucocytozoon* spp. being primarily transmitted by black flies, *Haemoproteus* spp. by mosquitoes and biting midges, and *Plasmodium* spp. by mosquitoes (Atkinson and van Riper 1991; Valkiunas 2005). Consequences of infection with these parasites reported in birds range from seem-ingly benign (Kilpatrick et al. 2006; Bensch et al. 2007; LaPointe et al. 2012) to impacts on reproductive fitness (Merino et al. 2000; Marzal et al. 2005; Asghar et al. 2015) and survival (Herman et al. 1975; Atkinson et al. 2000; Asghar et al. 2015).

Although haemosporidian infection in birds is common (Clark et al. 2018; Ellis et al. 2019), novel introductions of such parasites into naive avian populations have been documented and may be associated with population-level impacts (Warner 1968; van Riper et al. 1986). Thus, global climate change has the potential to facilitate future biologically consequential introductions of haemo-

sporidian parasites into naive avian populations, through shifts in the ranges of parasites and their vectors to higher latitudes and elevations (Sekercioglu et al. 2008; Chen et al. 2011; Fecchio et al. 2019), lengthened breeding seasons of arthropod vectors, and improved conditions for parasite reproduction (LaPointe et al. 2010; Zamora-Vilchis et al. 2012; Loiseau et al. 2013). Additionally, changes in environmental conditions may alter the abundance of parasites at locations where they are currently uncommon (Brooks and Hoberg 2007). Such events may be more likely to occur in high-latitude regions, where the effects of climate change have been most pronounced (Kutz et al. 2009).

The Yukon-Kuskokwim Delta (YKD), a globally important wetland complex where more than a million geese congregate annually to breed, lies in one such high-latitude region within western Alaska, US. Three species of geese sympatrically breeding on the YKD have previously been sampled for haemosporidian parasites: Emperor Geese (*Anser canagicus*), Cackling Geese (*Branta hutchinsii minima*), and Black Brant (*Branta bernicla nigricans*). A single *Leucocytozoon* spp. infection was identified from 134 Emperor Goose (<1%) blood smears collected on the YKD in 1996 (Hollmén et al. 1998) in contrast to a higher prevalence estimate (19.9%, 95% confidence interval [CI]: 3.0–36.8%) for parasite infections in this same species and location, as inferred from molecular screening of whole blood samples collected during 2011–12 (*n*=77; Ramey et al. 2014). In the same investigation using molecular techniques, *Leucocytozoon* spp. infections were also detected in Black Brant (11.1%, 95% CI: 0–29.5%) sampled on the YKD during 2011–12 (*n*=49) but not in Cackling Geese sampled at this same locale in 2011 (*n*=117). Thus, previous sampling of geese on the YKD for haemosporidian parasites suggests that there may be considerable variation in prevalence among sympatrically nesting geese.

In this study, we further assessed the detection and prevalence of avian haemosporidian parasites in Emperor and Cackling Geese breeding on the YKD. Specifically, we used consistent methods to examine whether: 1) Emperor Geese sampled in 1998 had a different incidence of haemosporidian parasitic infections compared to those sampled in 2014, 2) Emperor Geese harbor a higher prevalence of haemosporidian parasites than do Cackling Geese raising broods in the same geographic area, and 3) transmission is potentially occurring within the YKD. Results of this study help to elucidate variation in haemosporidian parasite prevalence among sympatrically breeding geese on the YKD, which may inform future assessments of change in parasite distribution and abundance.

MATERIALS AND METHODS

We obtained blood samples from Emperor Geese during incubation and brood rearing in 1998 and 2014, and from Cackling Geese during brood rearing in 2014, at locations adjacent to the Manokinak River (approx. 61°N, 165°W) on the YKD. Bow nets or mist nets were used to capture incubating adult female Emperor Geese. Flightless hatch year and adult geese were captured during brood rearing by herding birds into corrals. Capture and handling protocols were reviewed by the Animal Care and Use Committee at the US Geological Survey (USGS) Alaska Science Center and authorized under USGS federal bird banding permit no. 20022. Sex and age class (hatch year vs. adult) for both species were determined by plumage and cloacal examination. Whole blood was collected via jugular venipuncture and stored in Longmire buffer. Samples were kept at ambient temperature for up to ~12 h before freezing, then remained frozen until molecular analysis.

We extracted DNA from whole blood using a DNeasy Blood and Tissue kit (Qiagen, Valencia, California, USA) following the manufacturer's protocol. To confirm successful DNA extraction, a 481-base pair fragment of the mitochondrial DNA (mtDNA) cytochrome oxidase I gene of the avian host was amplified using primers previously published in Ramey et al. (2014) and thermal-cycling conditions reported by Kerr et al. (2007). Each DNA extraction was screened three times for the presence of haematozoa using a nested PCR protocol described by Hellgren et al. (2004). A minimum of one negative control for every 24 wells was incorporated into each set of PCRs. Reactions were conducted in eight-well strip tubes with individual caps that remained closed except while loading template and reagents to

prevent cross contamination. Primer sequences and amplification protocols are briefly summarized in Supplementary Material File S1. Amplicons were visualized on 0.8% agarose gels stained with Gel Red Nucleic Acid Gel Stain (Biotium, Hayward, California, USA).

Products from the PCR appearing to represent the 479-base pair cytochrome b (cyt b) mtDNA target were treated with ExoSap-IT (USB Inc., Cleveland, Ohio, USA) and sequenced with identical primers used for the PCR and with BigDye Terminator version 3.1 mix (Applied Biosystems, Foster City, California, USA) on an Applied Biosystems 3730xl automated DNA sequencer. Sequence data were cleaned and edited using DNA Dynamo (Blue Tractor Software Ltd., North Wales, UK). Sequences were then assigned to haemosporidian parasites of three genera (*Leucocytozoon*, *Haemoproteus*, or *Plasmodium*) using the nucleotide BLAST function available through the National Center for Biotechnology Information (NCBI; 2021). Assignment was based on the top NCBI BLAST result with a minimum max identity score of 90%. A sample was identified as positive for *Leucocytozoon*, *Haemoproteus*, or *Plasmodium* spp. parasites if any of the three replicate PCRs resulted in a bidirectional double-stranded target product that was verified through genetic sequencing. Samples from which single-stranded or otherwise ambiguous products resulted were considered negative. Information on cyt b mtDNA haplotypes are available in Reed et al. (2021) and NCBI GenBank (accession nos. MW574916–MW574929).

Given imperfect parasite detection (i.e., sensitivity) using our PCR-based approach, we employed occupancy modeling (MacKenzie et al. 2017) to provide estimates of detection and prevalence. We used the program MARK (White and Burnham 1999) through the RMARK package (Laake 2013) in the program R (version 4.0.2; R Core Team 2020) to build models and address the two primary study objectives (i.e., assessing temporal variation and differences between sympatric species in detection and prevalence of parasites) with separate analyses. Due to small sample size, we limited our model sets to all additive combinations of variables for prevalence (Ψ) while holding detection (ρ) to a single variable or constant. To be conservative in our inference, we did not include interactions between variables influencing prevalence (see Supplementary Material Tables S1, S2).

First, we focused on comparing results for Emperor Geese sampled in 1998 versus 2014. We assessed temporal variation in parasite detection and prevalence in Emperor Geese through a series of models incorporating covariates for age class (hatch year vs. adult), breeding stage (nesting vs. brood rearing), and year (1998 and 2014). Next, we compared results between Emperor Geese and Cackling Geese sampled in 2014. We assessed species-specific variation in detection and prevalence through a series of models incorporating covariates for species, age class, and sex. In both analyses, model fit was assessed to inform which model structures best fit the observed data. We used the Fletcher's c-hat values for the most general models in each analysis to evaluate goodness of fit and quantify appropriate variance inflation factors. These inflation values were used to create quasi-likelihood Akaike's information criterion corrected for small sample size ($QAIC_C$) for model selection. An information theoretic approach was used to determine influences on parasite prevalence and detection. Models were ranked by $\Delta QAIC_C$ scores and quasi-Akaike weights. The $\Delta QAICc$ scores were calculated as the difference between each model and the most parsimonious model. Detection and prevalence estimates were obtained by interpreting the most parsimonious model, unless one did not emerge, then model averaging was used to obtain unbiased point estimates and confidence intervals.

RESULTS

We extracted DNA from 307 blood samples collected from geese at sites on the YKD during 1998 (*n*=72 Emperor Geese) and 2014 (*n*=125 Emperor Geese, *n*=110 Cackling Geese) and molecularly detected *Leucocytozoon*, *Haemoproteus*, and *Plasmodium* spp. parasites (Table 1). Parasites of all three genera were identified in hatch year birds captured during brood drives (Table 1). The majority (45/57, 79%) of haemosporidian infections molecularly identified were assigned to the genus *Leucocytozoon*; therefore, the small numbers of *Haemoproteus* (*n*=7) and *Plasmodium* (*n*=6) detections were excluded from modeling efforts.

In our first analysis to assess temporal variation in parasite detection and prevalence between Emperor Geese sampled in 1998 and 2014, we detected moderate lack of fit in the most general model incorporating covariates for detection by sample year and for prevalence by sample year, breeding stage, and age class (Table S1). Therefore, a variance inflation factor of 1.67 was applied to the entire set of models and parameter estimation. Given

TABLE 1.   Summary of molecular screening of whole blood samples collected from Emperor and Cackling Geese (*Anser canagicus* and *Branta hutchinsii minima*) breeding on the Yukon-Kuskokwim Delta of Alaska, USA in 1998 and 2014 for haemosporidian parasites of the genera *Leucocytozoon*, *Haemoproteus*, and *Plasmodium* by year, breeding stage, age class, and sex class.[a]

Species	Year	Breeding stage	Age class	Sex	n	*Leucocytozoon* positive, n (%)	*Haemoproteus* positive, n (%)	*Plasmodium* positive, (%)
EMGO	1998	Nesting	Adult	F	20	5 (25)	0	0
EMGO	1998	Brood drives	Adult	F	18	4 (22)	1 (6)	0
EMGO	1998	Brood drives	Adult	M	13	6 (46)	0	0
EMGO	1998	Brood drives	Hatch year	F	11	4 (36)	0	0
EMGO	1998	Brood drives	Hatch year	M	10	2 (20)	0	0
EMGO	2014	Nesting	Adult	F	28	6 (21)	0	0
EMGO	2014	Brood drives	Adult	F	36	9 (25)	1 (3)	0
EMGO	2014	Brood drives	Adult	M	9	0	0	0
EMGO	2014	Brood drives	Hatch year	F	24	1 (4)	2 (8)	0
EMGO	2014	Brood drives	Hatch year	M	28	5 (18)	0	1 (4)
CACG	2014	Brood drives	Adult	F	27	0	1 (4)	1 (4)
CACG	2014	Brood drives	Adult	M	22	2 (9)	0	2 (9)
CACG	2014	Brood drives	Hatch year	F	29	1 (3)	1 (3)	2 (7)
CACG	2014	Brood drives	Hatch year	M	32	0	1 (3)	0

[a] EMGO = Emperor Geese; F = female; M = male; CACG = Cackling Geese.

the absence of a clear top model (Table S1), we used model averaging to attain unbiased point estimates and confidence intervals. Using this approach, we found model averaged point estimates of *Leucocytozoon* prevalence ranging from 17.8% (95% CI: 9.4–31.1%) for goslings in 2014, to 26.9% (95% CI: 14.0–45.3%) for incubating females in 1998 (Fig. 1). We also found little support for differences in the detection of *Leucocytozoon* parasites in Emperor Geese sampled in 1998 versus those sampled in 2014, or for variation in parasite detection and prevalence by breeding stage and age class (Fig. 1 and Table S1). Estimates of detection probability ranged between 0.72–0.77 (95% CI: 0.53–0.88) per PCR run or 0.90–>0.99 per sample when run in triplicate.

In the second analysis, to examine species-specific variation in parasite detection and prevalence, we again detected moderate lack of fit in the most general model incorporating covariates for detection by species and prevalence by species, age class, and sex (Table S2). Therefore, a variance inflation factor of 1.58 was applied to the entire set of models and parameter estimation. However, model averaging was not necessary in this analysis to obtain point estimates and confidence intervals because a clear best model was identified, incorporating covariates for detection by age class and prevalence by species (QAICc weight = 0.41; Table S2). This top model received approximately three times the weight of the next-best ranked model. Furthermore, models including species as a covariate for prevalence received over 80% of total model weight in the candidate set. The prevalence estimates from our top model were 20.3% (95% CI: 11.8–32.7%) for Emperor Geese and 3.6% (95% CI: 1.1–11.0%) for Cackling Geese (Fig. 1). We found covariates for age class and sex to be uninformative for prevalence. However, four of the five top models (>75% of model weight in the set) included age class as a detection covariate (Table S2). The detection estimates from our top model were 0.85 (95% CI: 0.67–0.94) for adult geese and 0.24 (95% CI: 0.10–0.80) for goslings per PCR run (Fig. 1). This equates to estimated detection probabilities of >0.99 and 0.56 for samples from adult and hatch year geese, respectively, when run in triplicate.
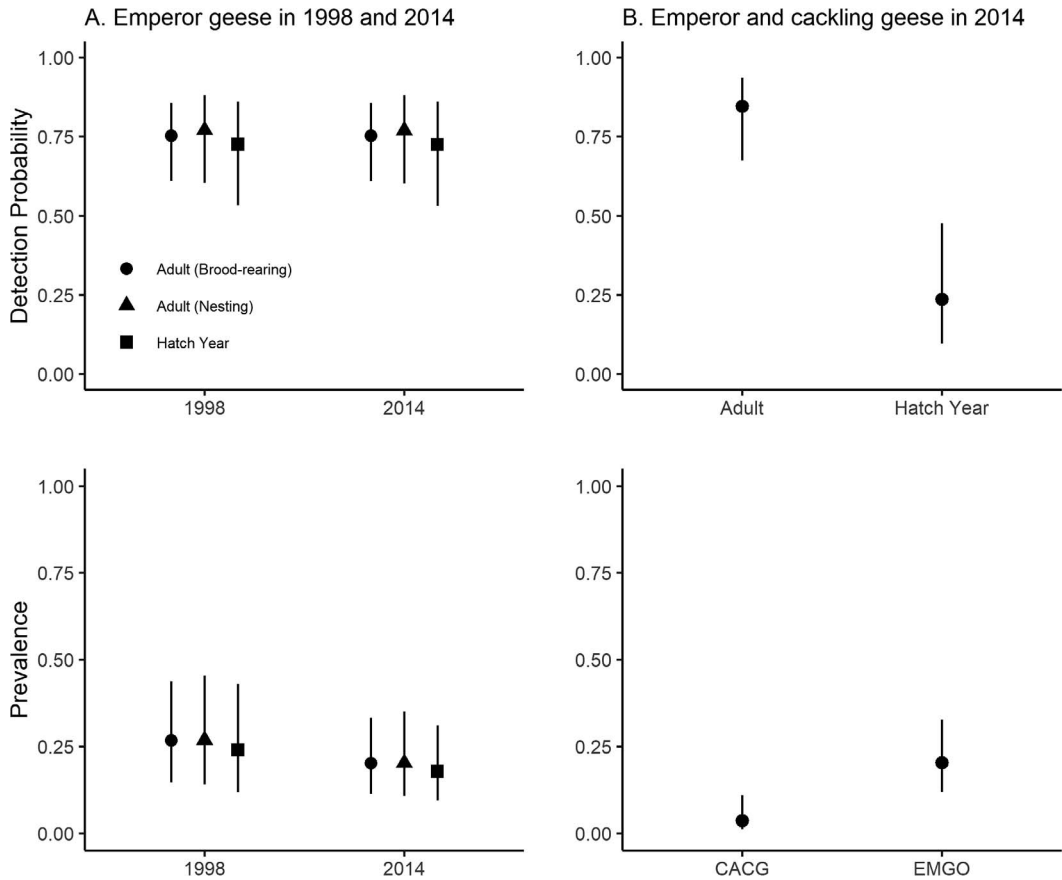
FIGURE 1.   Detection (per replicate PCR run) and prevalence estimates with 95% confidence intervals for *Leucocytozoon* spp. parasites in: A. Emperor Geese (*Anser canagicus*) breeding on the Yukon-Kuskokwim Delta of Alaska, USA sampled during the incubation and brood-rearing periods in 1998 and 2014; and B. Emperor (EMGO) and Cackling (CACG) Geese (*Branta hutchinsii minima*) sympatrically breeding on the Yukon-Kuskokwim Delta sampled during the brood-rearing period in 2014 only.

DISCUSSION

Results of our investigation provide important insights regarding variation in the detection and prevalence of blood parasites among sympatrically breeding geese on the YKD in western Alaska. Specifically, our results provide evidence that Emperor Geese have probably consistently harbored *Leucocytozoon* spp. infections through recent time; that sympatrically breeding Emperor Geese and Cackling Geese may differentially harbor *Leucocytozoon* spp. infections; and that hatch year geese on the YKD may harbor a diversity of haemosporidian parasite infections that may not be as readily detected using PCR as

they are in adult birds. These findings help to establish an important baseline for future assessments of changes in parasite prevalence and potential population-level effects in response to predicted ecologic change.

Using blood samples obtained from additional breeding seasons, our data are congruent with previous molecular screening efforts that support the consistent infection of Emperor Geese breeding on the YKD with *Leucocytozoon* spp. at a prevalence of around 20% (Ramey et al. 2014). We note that slight (nonsignificant) differences in point estimates of *Leucocytozoon* spp. prevalence for Emperor Geese in 2014 in two analyses as part of this study are due to model averaging in the first

analysis, using Emperor Goose samples collected in 1998 and 2014. In our second analysis, we included only samples collected in 2014 from both Emperor and Cackling Geese. Given relatively consistent evidence for the prevalence of *Leucocytozoon* spp. among Emperor Geese at all time points assessed using molecular methods (this study and Ramey et al. 2014), we infer that the apparent increase in *Leucocytozoon* spp. prevalence since the first record by Hollmén et al. (1998) in 1996 is most likely a function of methodology. In this case, the increased sensitivity of modern molecular methods probably accounted for the higher *Leucocytozoon* spp. prevalence estimates in recent investigations as opposed to an actual increase in parasite prevalence since 1996. Conclusions regarding trends of infection through time could be strengthened through testing of Emperor Goose blood samples from additional years.

Also consistent with a previous report (Ramey et al. 2014), we found evidence that Emperor Geese may indeed differentially harbor *Leucocytozoon* spp. infections compared to sympatrically breeding Cackling Geese. In our analysis, the prevalence of *Leucocytozoon* spp. infections in Emperor Geese was estimated to be more than five times that of Cackling Geese sampled on the YKD, despite these species nesting and rearing broods in the same geographic area. Differential parasite prevalence among sympatrically breeding species has also been observed in other systems where closely related taxa inhabit different wintering areas (Yohannes et al. 2009; Emmenegger et al. 2018). In the case of geese inhabiting the YKD, Cackling Geese tend to use agricultural fields within temperate lower latitudes during the nonbreeding season (Bogiatto et al. 2009) as opposed to Emperor Geese, which are dependent on natural food sources within subarctic high-latitude locations throughout the wintering period (Hupp et al. 2008; Uher-Koch et al. 2021). Consequently, Cackling Geese may more readily procure food resources and attain better physical condition prior to the onset of breeding; this might

facilitate a robust immune response sufficient to quickly resolve haemosporidian infections (Arriero et al. 2018). In contrast, Emperor Geese may not arrive on the YKD with ample energetic reserves to meet the combined physiologic demands of breeding (i.e., egg laying and incubation) and mounting a sufficient immune response to suppress parasitic infections to undetectable levels. Alternatively or additionally, geographic variation in environmental conditions (Fecchio et al. 2020), or some degree of host specificity of parasites or vector preferences of biting insects (Hellgren et al. 2004), may play a role in parasite epidemiology on the YKD. Regardless of the underlying mechanism(s), our results suggest that avian haemosporidian parasites have the potential to disproportionally affect Emperor Geese breeding on the YKD; therefore, research to assess detrimental effects of *Leucocytozoon* spp. infections in this species is warranted.

The molecular identification of *Leucocytozoon*, *Haemoproteus*, and *Plasmodium* spp. parasites in unflighted goslings on the YKD provides important baseline information for future assessments of ecologic change, as this finding supports contemporary transmission of all three parasite genera at this locale. The finding of few *Haemoproteus* and *Plasmodium* spp. detections in waterfowl inhabiting the YKD is consistent with prior reports (Ramey et al. 2014; Ramey et al. 2015) and the premise that parasites of these genera may be temperature limited in high-latitude regions (Clark et al. 2020). Support for a difference in the estimated molecular detection of *Leucocytozoon* spp. in adult versus gosling Emperor Geese in our second analysis was unexpected, particularly given that this detection difference was not clearly implicated in our first analysis focused on Emperor Geese nor in previous models assessing haemosporidian parasite detection in samples collected from geese (Ramey et al. 2014), ducks (Ramey et al. 2015), and crows (Van Hemert et al. 2019) inhabiting Alaska. It is possible that the difference in detection we found in our analysis may have been due to unidentified sampling artifacts (e.g., disproportional sam-

pling of birds exhibiting low parasitemia) among the relatively small sample size of *Leucocytozoon* spp.-positive hatch year birds assessed in our analytical framework ($n=7$). We encourage future investigations to incorporate age as a covariate when assessing variance in parasite detection until biologic significance is resolved.

In summary, our investigation establishes important baseline information regarding variation in the detection and prevalence of blood parasites that will be useful for informing future assessments of parasite prevalence in geese breeding in western Alaska and for evaluating potential ecologic effects in response to predicted environmental change. For example, our investigation provides additional support that *Leucocytozoon* spp. have clearly been established in geese breeding in western Alaska for at least 2 decades and therefore do not represent an emergent parasitic threat in the region. However, it is still unclear if haemosporidians are causing biologically significant effects in geese breeding on the YKD at the population level, which is worthy of future assessment. Furthermore, our study supports the previous finding that *Haemoproteus* and *Plasmodium* spp. parasites are uncommon among breeding waterfowl on the YKD but are transmitted within this region. Therefore, these presumably temperature-limited parasites may represent valuable targets for assessing future ecologic change within the YKD.

ACKNOWLEDGMENTS

SUPPLEMENTARY MATERIAL

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