

*Exxon Valdez* Oil Spill  
Restoration Project Annual Report

A Genetic Study to Aid in Restoration of Murres, Guillemots and Murrelets to the Gulf of  
Alaska

Restoration Project 98169  
Annual Report

This annual report has been prepared for peer review as part of the *Exxon Valdez* Oil spill  
Trustee Council restoration program for the purpose of assessing project progress. Peer  
review comments have not been addressed in this annual report.

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April 1999

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# **A Genetic Study to Aid in Restoration of Murres, Guillemots and Murrelets to the Gulf of Alaska**

## **Restoration Project 98169 Annual Report**

**Study History:** In the Final Report on project 96-038, the Pacific Seabird Group suggested that genetic variation within and among populations of common murres, pigeon guillemots, and marbled and Kittlitz's murrelets from the Gulf of Alaska and surrounding regions be examined both to assess the impact of the *Exxon Valdez* Oil spill on these species and to aid in their restoration to the Gulf of Alaska. Restoration Project 97169 was initiated in FY97 to examine the genetic structure of populations of murres, guillemots and murrelets in the North Pacific. Annual Report 97169 documented results of sample collections and laboratory analyses for research initiated under Restoration Project 97169. The project was continued under Restoration Project 98169. Work under Restoration Project 99169 (the third of four stages) currently is underway, and a proposal is being submitted for the fourth and final year of research under Restoration Project 00169. One manuscript based on data collected under this project has been submitted to *Evolution* and another is in preparation. This is the second annual report for research initiated under Restoration Project 97169.

**Abstract:** DNA sequence variation in mitochondrial DNA, microsatellite DNA and nuclear introns is being assayed to aid restoration of common murres (*Uria aalge*), pigeon guillemots (*Cepphus columba*), marbled murrelets (*Brachyramphus marmoratus*) and Kittlitz's murrelets (*B. brevirostris*) to the Gulf of Alaska. In FY97 and FY98, tissue and blood samples were collected from all four species from several sites within the Spill area and adjacent sites. In FY97, protocols for screening variation in the mitochondrial control region were refined for murres, and techniques for assaying variation in nuclear introns and microsatellites were perfected for murres. Variation in nine introns and three microsatellites was assayed in marbled murrelets, and variation in the mitochondrial control region and cytochrome *b* gene was assayed in common murres. In FY98, protocols for assaying variation in introns in guillemots were perfected, and guillemot samples were screened for variation in five introns and one microsatellite locus. Common murres were screened for variation in the mitochondrial control region, five microsatellite loci and three introns, and murrelets were assayed for variation at one additional intron. Preliminary analyses indicate that this approach should provide colony-specific markers, and that hybridization occurs between common and thick-billed murres (*U. lomvia*). No restoration recommendations can be made yet.

**Key Words:** common murre, cytochrome *b*, cryptic species, introns, gene flow, Kittlitz's murrelet, marbled murrelet, microsatellites, mitochondrial control region, pigeon guillemot, source and sink populations, SSCPs

**Project Data:** Data collected to date include frequencies and sequences of intron alleles and mitochondrial haplotypes and frequencies of microsatellite alleles for common murre, pigeon guillemots, marbled murrelets, and Kittlitz's murrelets. Preliminary estimates of population genetic structure and gene flow, and phylogenetic hypotheses for mitochondrial genotypes have been derived. All data are preliminary, and should not be used or cited without prior written permission of the authors.

**Citation:** Friesen, V.L. and J.F. Piatt. 1999. A genetic study to aid in restoration of murre, guillemots and murrelets to the Gulf of Alaska. *Exxon Valdez Oil Spill Restoration Project Annual Report (Restoration Project 98169)*, Alaska Biological Science Center, U.S. Geological Survey, Anchorage, Alaska.

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## **Executive Summary**

We are using state-of-the-art molecular methods to aid in the restoration of common murres, pigeon guillemots, and marbled and Kittlitz's murrelets to the Gulf of Alaska. For each species, we have three main objectives: (1) to determine the geographic extent of the populations affected by the Spill; (2) to identify source and sink colonies; and (3) to identify appropriate reference or 'control' sites for monitoring. To meet these objectives, we are comparing variation in two mitochondrial genes (the control region and cytochrome *b*), 6-8 microsatellite loci and 8-10 nuclear introns among approximately 30 birds from each of 12-15 colonies for each species (except for Kittlitz's murrelets, which are being sampled opportunistically). Results will be used to estimate the extent of genetic differentiation and gene flow among colonies, as well as genetic variability and inbreeding within colonies. DNA samples are being obtained primarily by J.F.P., and molecular analyses are being performed in V.L.F.'s laboratory at Queen's University.

We have just completed the second year of this project. Tissue samples were obtained from several new sites for all species. All available guillemot samples were screened for sequence variation in five introns and one microsatellite locus. New samples from common murres were screened for variation in the mitochondrial control region, and all available murre samples were screened for variation at five microsatellite loci and three introns. All available murrelet samples were assayed for variation at one new intron. Preliminary analyses suggest that gene flow among colonies of common murres is high, and that the present approach should provide colony-specific markers (in the form of allele frequency differences at a number of loci) for identifying the sources of oiled birds. No restoration recommendations can be made at this time.

## **Introduction**

Seabirds of the family Alcidae are highly vulnerable to marine oil pollution due both to the large amount of time they spend resting on the ocean surface, and to their dependence on marine fish and invertebrates for food. Many species of alcids suffered heavy mortality associated with the Spill; for example, the estimated mortality for common murres was in the hundreds of thousands. Although guillemots and murrelets were declining prior to the Spill, the accident probably increased their rate of decline. Common murres now appear to be recovering from the Spill, but pigeon guillemots and marbled murrelets apparently are not; the state of recovery of Kittlitz's murrelets is unknown. The reasons for the failure of these species to recuperate (as well as for the pre-spill declines) are unclear, but may relate to availability and quality of prey (currently being investigated through the APEX Predator Experiment and Nearshore Vertebrate Predator Project), and/or genetic problems such as genetic isolation of colonies or inbreeding. We are using state-of-the-art genetic techniques to aid in the restoration of these species.

Although the application of molecular methods to fisheries and wildlife management is common (e.g. Ryman and Utter 1987, Hansen and Loeschcke 1994, Allendorf and Waples 1996, Graves 1996), few if any studies have used genetic methods explicitly to aid in seabird conservation

(Friesen 1997). Theoretically, measurement of genetic divergence and gene flow among populations of murrelets, murrelets and guillemots will aid restoration in the following three main ways:

*Definition of the geographic limits of the affected populations.*-Many seabirds killed by the Spill were migrating: the 'affected' zone, or the populations that were affected by the Spill and require restoration effort, may be geographically distant from the actual Spill zone. Genetic data should enable identification of breeding populations of birds killed by the Spill. Furthermore, genetic data should indicate if colonies are essentially panmictic and/or constitute metapopulations, in which case they should recover without assistance within a few generations. However, if colonies constitute numerous localized populations, they may not naturally recolonize sites affected by the Spill, and may require human assistance for recovery.

*Identification of sources and sinks.*-According to metapopulation theory, 'source' populations are populations that occur in optimal habitat and can act as exporters of recruits for populations elsewhere; 'sink' populations occur in suboptimal habitat and require immigration to maintain numbers (e.g. Pulliam 1996). Genetic data can provide measurements of gene flow (including confidence limits)-into and out of colonies, and thus enable identification of sources and sinks. For example, protein data suggest that rock shags (*Stictocarbo magellanicus*) on the Falkland Islands may have served as the main source of breeders for other colonies in southern South America (Siegel-Causey 1997). If colonies affected by the Spill represent sources, then their restoration will be critical. If a colony represents a sink, its restoration may be a waste of resources and may actually prevent recovery of the species.

*Environmental monitoring.*-Demographic parameters may be very different for genetically divergent populations, even if they occur in ecologically similar or geographically proximate areas. For example, common murrelets breeding in Washington have different breeding chronologies from those at neighboring colonies in British Columbia, and may be genetically different (Warheit et al. unpubl. data). Genetic data may enable identification of appropriate reference or 'control' sites from which to obtain baseline data for monitoring, restoration and modeling, e.g. to determine if a seabird colony has recovered 'normal' functioning.

Three other types of information that are useful for conservation and restoration are produced incidentally by genetic studies:

*Population uniqueness and cryptic species.*-A colony's uniqueness (e.g. its endemism or genetic distinctiveness) may be used to prioritize restoration efforts. Most importantly, genetic data enable the identification of cryptic species - populations that are similar in appearance but that represent separate, non-interbreeding species (e.g. long-billed [*Brachyramphus perdix*] and marbled murrelets; Friesen et al. 1996a).

*Small effective population size and inbreeding.*-The longterm effective size of a population is the size of an idealized population that would have the same amount of genetic diversity as the

population being considered; the longterm effective size of a population may be one or two orders of magnitude lower than its census size due to such factors as unequal breeding success and population bottlenecks (Futuyma 1998). For example, the North Atlantic population of thick-billed murres (*Uria lomvia*) consists of approximately 2.5 million breeding pairs (Nettleship and Evans 1985), but appears to have a longterm effective size of only ~15,000 females (Friesen et al. 1996b). Theoretically, as a population's effective size decreases, individual fitness declines due to increased inbreeding (Allendorf and Leary 1986, Gilpen and Soulé 1986); several researchers have argued that if effective population size declines below a certain critical level, the population may enter an extinction vortex in which inbreeding, deleterious alleles and stochastic effects combine synergistically to accelerate extinction (Gilpin and Soulé 1986). Application of a new body of theory known as Coalescence Theory to genetic information may be used to estimate longterm effective population size and to place confidence limits on these estimates (Beerli 1999 and references therein). Thus the extent to which small effective population sizes and inbreeding are preventing or slowing population recovery may be inferred.

*Translocations.*-If breeding success within a colony is low due to inbreeding depression, or if recruitment is low, transplantation of small numbers of individuals from other sites may be desirable. Ideally, sources of animals for such introductions should be neighboring colonies within the same population or a closely related population. Genetic data are important for determining which colonies are genetically appropriate sources to prevent both inbreeding (Allendorf and Leary 1986) and outbreeding depression (Templeton 1986).

## **Objectives**

The primary purpose of this project is to conduct genetic analyses to aid in the restoration of common murres, pigeon guillemots, and marbled and Kittlitz's murrelets to areas affect by the Spill. We have three main objectives for each species:

- 1) to determine the geographic extent of the populations affected by the Spill;
- 2) to identify source and sink colonies; and
- 3) to identify appropriate reference or 'control' sites for monitoring.

As incidental results, we should also be able

- 4) to identify cryptic species or subspecies,
- 5) to measure coefficients of inbreeding and longterm effective population sizes, and
- 6) to identify appropriate source populations for translocations, if necessary.

## Methods

We are comparing variation in two mitochondrial genes, 6-8 microsatellite loci and 8-10 nuclear introns among approximately 30 birds from each of 12-15 colonies for each species except Kittlitz's murrelets, for which samples are difficult to obtain (Table 1). For each species, we are testing the null hypothesis that colonies are panmictic (genetic structure is essentially absent) against the alternative hypothesis that significant genetic differences exist among birds from different colonies.

*Sampling.*-To obtain reliable estimates of genetic differentiation and gene flow within the Spill area and between the Spill area and neighboring sites, as well as to define the geographic limits of the breeding populations, we are sampling 4-6 colonies of each species from the spill area, as well as 4-6 colonies each west and east of the Spill area. A minimum of 30 samples are required from each site for each species for reliable estimation of genetic variation within and between sites (Richardson et al. 1986, Weir 1996). Many of the necessary baseline samples were obtained opportunistically during previous projects through the assistance of other researchers.

*Loci.*-Much of southern Alaska was ice-covered during the Pleistocene glaciations, so most seabird colonies from the Spill area were probably only populated within the last ~10,000 years. Measurement of gene flow and genetic divergence among colonies of these birds therefore requires analysis of loci with high mutation rates. Mitochondrial DNA (mtDNA) has proven useful for studies of such populations since it has a relatively high mutation rate and is more sensitive to population bottlenecks and restricted gene flow than are nuclear loci (Wilson et al. 1985, Avise 1994, Avise and Hamrick 1996, Mindell 1997). The mitochondrial control region is especially useful for analyzing recently isolated populations since it has a mutation rate 5-10x higher than the mean for mtDNA (Brown et al. 1986, Avise 1994, Avise and Hamrick 1996, Baker and Marshall 1997). The mitochondrial cytochrome *b* gene also is useful for estimating population genetic structure and longterm effective population sizes in alcids since its mutation rate has been calibrated for this family (unpubl. data). However, mtDNA represents a single supergene whose pattern of inheritance is not typical of the rest of the genome (Wilson et al. 1985); results of analyses of mtDNA therefore need to be confirmed with analyses of nuclear loci. Microsatellite loci have mutation rates higher than those of mtDNA so are being used increasingly for evolutionary studies (Avise 1994, Dowling et al. 1996, McDonald and Potts 1997). However, depending on the age of populations, microsatellite loci may contain high levels of homoplasies (back-, parallel and convergent mutations), which may result in inaccurate estimates of genetic differentiation and gene flow. Nuclear introns have mutation rates equivalent to those of mtDNA (unpubl. data), so are also useful for studying recent evolutionary events (Friesen et al. 1996; Congdon et al. submitted). Because microsatellites and introns are nuclear loci, they are less sensitive to population bottlenecks and restricted gene flow than are mitochondrial genes; Moore (1995) estimated that, due to the larger effective population size of nuclear genes, 8-16 nuclear loci are required to obtain information equivalent to that of one mitochondrial gene. Previous researchers (e.g. Richardson et al. 1986, Weir 1996) have also

suggested that information from at least five to six nuclear loci are required to obtain reliable estimates (i.e. to derive robust error estimates) of genetic structure and gene flow. Thus, we are analyzing the mitochondrial control region and cytochrome *b* gene, as well as 8-16 nuclear loci, with the specific number of each class of marker depending on observed levels of variability and gene flow.

*Laboratory Assays.*-Variation in the number of repeating units in microsatellite loci is being assayed using standard protocols (Dowling et al. 1996). To reduce time and cost associated with assaying sequence variation in mitochondrial genes and introns, a two-step procedure is being used. Samples are first screened for mutations using analysis of single-stranded conformational polymorphisms (SSCPs; Friesen et al. 1996a, 1997). The exact nature of mutations is then determined by direct sequence analysis of at least one individual with each genotype detected from SSCP. Previous experience indicates that this combination of techniques provides an efficient and sensitive method for comparing sequence variation among populations (Friesen et al. 1996a, 1997, Congdon et al. submitted). We estimate that a trained technician can process approximately 4500 sample-loci per year. Analysis of 20 loci (two mitochondrial genes, eight microsatellite loci and ten introns) for each of approximately 1200 samples (excluding 150 murrelets already analyzed by Congdon et al.) is expected to require approximately 5.5 person-years.

*Statistical Analyses.*-Data are being analyzed using standard methods developed for data from protein electrophoresis and sequencing (e.g. Swofford & Selander 1981; Swofford 1993), as well as using new techniques that capitalize on the power of combining genotypic and sequence data (e.g. Michalakis and Excoffier 1996, Beerli 1999):

- 1) To determine the geographic limits of populations affected by the Spill, the extent of genetic differentiation of colonies is being calculated using Wright's *F* statistics and its analogues (e.g.  $\phi_{st}$ ) and tested for significance using randomization procedures (e.g. Excoffier et al. 1992).
- 2) To identify source and sink colonies, the direction and magnitude of gene flow (including confidence limits) among colonies is being estimated using coalescence theory (Slatkin and Maddison 1989, Beerli 1999).
- 3) Appropriate reference or 'control' sites for monitoring, will be apparent from the results of objective (1); colony-specific markers (in the form of allele frequency differences at multiple loci) for impact assessment will be determined using SPAM (ADFG 1999) and Assign (M. Damus, unpubl. program).
- 4) Cryptic species will be inferred from (i) fixed allele differences, which indicate prolonged genetic isolation of populations, (ii) paraphyletic relationships among populations from different species, and/or (iii) high sequence divergences between the mitochondrial genomes of individuals from different populations.

- 5) Coefficients of inbreeding will be estimated from nuclear data using Wright's F statistics, and longterm effective population sizes (including confidence limits) will be estimated from mitochondrial sequence data using the method of Beerli (1999), which is based on Coalescence Theory.
- 6) Appropriate source populations for translocations will be apparent from the results of objective (1).

## Results

This project requires collection of blood, feather and/or tissue samples from birds breeding throughout the Pacific basin, mostly in Alaska (Table 1). As much as possible, tissue is being obtained from museum specimens, and blood and blood feathers ('pin' or growing feathers) is being obtained from chicks or adults during banding. Birds being collected for ongoing dietary studies in Alaska (J.F.P.) also are being used for tissue. In FY98 we obtained samples from common murrelets from the eastern Aleutians, from marbled murrelets from the central and eastern Aleutians, and from guillemots from British Columbia and Kachemak Bay. Most samples are being obtained through contributions from researchers working at specific sites, but a special collection trip was made to British Columbia and Kachemak Bay (for guillemots). Sampling efforts in 1999 will focus on remaining key sites (Table 1).

We have just completed the second year of this project. Turnover in personnel working on both the guillemots and the murrelets meant that fewer samples were screened than planned; however, this turnover also resulted in salary savings in FY97 which are being used to pay additional personnel in FY98. Thus, more effort will be made on screening these two species in FY00.

*Common Murres.*-Assays of sequence variation in the mitochondrial control region (treated as two loci for screening), microsatellite loci and introns was begun in FY97 and was continued in FY98. Preliminary analyses of sequence variation in mitochondrial DNA indicate that significant gene flow occurs among colonies of common murrelets. An unexpected finding was that six of 120 common murrelets sampled in the Gulf of Alaska carried mtDNA sequences of thick-billed murrelets and various combinations of nuclear alleles from common and thick-billed murrelets, suggesting that ~5% of common murrelets in this area are hybrids and back-crosses. (In a similar survey of thick-billed murrelets in the North Pacific, one was found to carry the mtDNA sequence of a common murre; M. Damus, unpubl.) Preliminary investigations of the utility of microsatellites in identifying the sources of common murrelets in simulated by-catch samples indicate that simulated samples could be ascribed with confidence to collection sites in British Columbia, Washington, California and Oregon (Warheit et al. unpubl. data). Thus, there is a good probability of finding colony-specific markers (in the form of allele frequency differences at a large number of loci) for murrelets from the Gulf of Alaska using the present approach.

*Marbled and Kittlitz's Murrelets*.-Samples from marbled murrelets were screened for sequence variation at a tenth intron (an MHC intron). Preliminary investigations indicate that there is a good probability of finding colony-specific markers (in the form of allele frequency differences at a large number of loci) for murrelets from the Gulf of Alaska using the present approach (Friesen et al., unpubl. data).

*Pigeon Guillemots*.-Samples for pigeon guillemots were screened for variation at five introns and one microsatellite locus. No analyses have been conducted to date.

## **Discussion**

Due to the preliminary nature of the present data, interpretation of results in terms of the primary objectives will be not addressed until the Final Report.

## **Acknowledgements**

Many samples used in this study were obtained through the assistance of Vernon Byrd, Dave Roseneau, S. Kitaysky, Jay Pitocchelli, Tom van Pelt and Lindsey Hayes, Alex Pritchard, Jan Hodder, Amy Marr, Alan Burger and Kathy Martin. Tim Birt, Brad Congdon, Christine Crossman, Shane Doran, Deb Harrison, Karen Holder, Gabriela Ibarguchi, Heather Jones, Kathy Kennedy, Monica Kidd, Vinay Lodha, Denise Michaud, Jeff Moy, Anoma Patirana, Lisa Veit and Jesse Wood provided technical help and valuable discussions. K. Warheit shared unpublished data and analyses.

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Table 1. Sites, numbers of samples available, and numbers of samples needed for genetic analyses of murre, murrelets and guillemots.

Site	Available	Needed
<b>COMMON MURRE</b>		
California (Farallon Islands)	30	0
Washington (Clallam)	30	0
N. Vancouver Island	40	0
Southeastern Alaska	0	30
Prince William Sound (Cordova)	23	7
Middleton Island	0	30
Central Cook Inlet (Kachemak Bay, Chisik I.)	48	0
Lower Cook Inlet (Barren Is.)	27	3
Central Alaska Peninsula (Semidi Is.)	24	6
Western Alaska Peninsula (Midun , Koniuji Is.)	18	12
Eastern Aleutians (Aiktak I.)	28	2
Western Aleutians (Attu, Agattu & Buldir Is.)	27	3
Bering Sea (Pribilof, St. Matthew, St. Lawrence Is.)	30	0
Chukchi Sea (Capes Lisburne & Thompson)	33	0
Sea of Okhotsk (Talan I., Magadanskaya)	30	0
Japan (Teuri I.)	0	30
<b>MARBLED MURRELET</b>		
California	40	0
Oregon	12	18
Washington	18	12
British Columbia (Queen Charlotte Is.)	30	0
Southeastern Alaska (Lemesurier I.)	20	10
Prince William Sound (Unakwik Fjord)	20	10
Cook Inlet (Kachemak Bay)	24	6
Kodiak Island	26	4
Mitrofanina Bay	26	4
Shumagin Islands (Koniuji Is., Belkofski B., Yakutat P.)	22	8
Eastern Aleutians (Dutch Harbor)	12	18
Central Aleutians (Adak I.)	10	20
Western Aleutians (Attu I.)	18	12

Table 1, cont'd.

Site	Available	Needed
<b>KITTLITZ'S MURRELET</b>		
Prince William Sound	4	*
Kachemak Bay	18	*
Adak Island	6	*
Western Aleutians (Attu I.)	5	*
Bering Strait	*	*
<b>PIGEON GUILLEMOT</b>		
California (Farallon Is.)	20	10
Oregon	25	5
British Columbia (Queen Charlotte Is.)	30	0
Southeast Alaska (Glacier Bay)	0	30
Prince William Sound (Jackpot & Naked Is.)	30	0
Cook Inlet (Kachemak Bay)	9	21
Kodiak Island	0	30
Alaska Peninsula (Semidi, Shumagin, Tanakiak Is.)	17	13
Western Aleutians (Attu, Agattu Is.)	0	30
Kuril Is.	0	30
Bering Sea (Pribilof, St. Lawrence Is.)	0	30
Chukchi Sea (Capes Thompson and Lisburne)	0	30

\*Samples will be obtained from Kittlitz's murrelets opportunistically.

NOTE: Every effort is made to obtain samples non-destructively to minimize the need for collections, e.g. as feathers or blood samples collected during banding, or from museum specimens.

## Products

The following papers, based on work funded entirely or in part by the EVOS Trustee Council, were submitted for publication in FY98.

Friesen, V.L. In press. Introns. *In* A. J. Baker (ed.), *Molecular Methods in Ecology*.

Friesen, V.L. and B.C. Congdon. In press. Intron variation and population genetics of birds. *Proceedings of the 22nd International Ornithological Congress*.

Friesen, V.L., B.C. Congdon, M.G. Kidd and T.P. Birt. General PCR primers for the amplification and sequencing of five nuclear introns in vertebrates. Submitted to *Molecular Ecology*.

Congdon, B.C., J.F. Piatt, K. Martin and V.L. Friesen. Rapid population expansion and peripheral isolation in marbled murrelets: contemporary vs historic processes. Submitted to *Evolution*.

Edwards, S.V., V.L. Friesen, M.C. Silva and K. Warheit. Tracing and demographic modeling of bycatch birds: the contribution of genetics. Submitted to the Proceedings of the 26th Annual Meeting of the Pacific Seabird Group Special Symposium on Seabird By-Catch.

The following conference papers, based on work funded entirely or in part by the EVOS Trustee Council, were presented in 1998.

Friesen, V.L. 1998. Intron variation and population genetics of birds. 22nd International Ornithological Congress. Symposium presentation.

Friesen, V.L., A. Patirana, T. Birt and M. Damus. 1998. Molecular evidence for hybridization between common and thick-billed murrelets in the North Pacific. 26th Annual Meeting of the Pacific Seabird Group. Poster presentation.

Congdon, B.C., K. Martin, J.F. Piatt and V.L. Friesen. 1998. Population genetics and conservation of marbled murrelets. Annual Meeting of the Society for Conservation Biology. Oral presentation.

Warheit, K.I., V.L. Friesen and T.P. Birt. 1998. Genetic structure among common murre populations from British Columbia to California. 26th Annual Meeting of the Pacific Seabird Group. Oral presentation.

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