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Mitochondrial Perspective on the Phylogenetic Relationships of the *Parula* Wood-warblers

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Studies of intraspecific genetic variation in Neotropical resident birds have frequently revealed a high degree of phylogeographic differentiation (e.g. Capparella 1988, Hackett and Rosenberg 1990, Peterson et al. 1992, Bates and Zink 1994, Hackett 1996). That trend suggests tropical bird species are particularly likely to be subdivided geographically and that their constituent lineages tend to be evolutionarily old. Only a few counterexamples have been reported in which molecular surveys of Neotropical avian species did not reveal phylogeographic structure. Brawn et al. (1996) found no differences in mtDNA RFLP haplotypes between populations of three passerine species on the Perlas Islands in the Bay of Panama and conspecific populations on the nearby mainland. However, the geographic distances (<100 km) and putative period of genetic isolation (<10,000 years) in that system were both short. Gutierrez (1994) found similarly low mtDNA divergence in Oilbirds (*Steatornis caripensis*) from colonies 400 km apart in northern Venezuela. On a broader geographic scale, Brumfield and Capparella (1996) observed considerable protein divergence between Central and South American House Wrens (*Troglodytes aedon*), but found little differentiation among locations along a 7,000 km transect from Panama to Tierra del Fuego.

Here we explore the magnitude of mtDNA differentiation among geographically distant populations of the Tropical Parula (*Parula pitiayumi*), and we use mtDNA sequences to explore the relationship of *P. pitiayumi* to the three other species currently placed in *Parula* (AOU 1998). *Parula pitiayumi* has a breeding distribution that extends from Northern Mexico south through much of South America and has been divided into 14 subspecies (Lowery and Monroe 1968). Based on its broad geographic distribution and high phenotypic diversity, our *a priori* expectation was that *P. pitiayumi* would display evidence of a high degree of phylogeographic variation in mtDNA. Instead, we found surprisingly modest mitochondrial variation both within *P. pitiayumi* and between that taxon and its congener *P. americana.* As described below, our mtDNA-based phylogenetic reconstructions suggest that *P. pitiayumi* and *P. americana* are conspecific, and reconstructions that included the other two *Parula* species, as well as representatives of several other wood-warbler genera, suggest that the four species currently placed in the genus *Parula* (AOU 1998) do not form a monophyletic group.

Methods.—Accession numbers, sources, and tissue collecting localities are given in Table 1. Our study included 15 *P. pitiayumi* individuals and two samples each of the other three currently recognized *Parula* species (*P. americana, P. gutteralis,* and *P. superciliosa*). Multiple representatives of two additional parulid genera (*Vermivora ruficapilla, V. peregrina, Dendroica adelaidae, D. tigrina*) thought to be closely allied to *Parula* (see AOU 1998:533) and a *Coereba flaveola* sample were also included in some analyses. DNA extraction, amplification, and sequencing procedures followed standard laboratory protocols described elsewhere (Lovette et al. 1998, 1999). We obtained the complete sequence (842 nucleotides) of the overlapping mitochondrial ATPase 8 and ATPase 6 genes from all individuals. From one or two individuals per species, we also obtained an additional 2,797 nucleotides of mtDNA sequence representing the complete cytochrome *b* and ND2 genes and 613 nucleotides of the cytochrome oxidase I gene. Cytochrome *b* sequences from *V. peregrina* were not included in analyses owing to the probable co-amplification of a nuclear-encoded pseudogene (Numt; I. Lovette unpubl. data) All sequences have been archived in GenBank (AFO18097, 18207, 256468–256519).

We used PAUP* 4.0b2 (Swofford 1999) to estimate genetic distances among individuals and to generate phylogenetic hypotheses. In analyses that included distantly related taxa, distances were estimated using the Hasegawa-Kishino-Yamura (HKY) metric (Hasagawa et al. 1985) with the transition : transversion ratio set to 6 and the gamma parameter set to

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TABLE 1. Collecting localities of specimens included in phylogenetic analyses. Tissue accession numbers and sources given in parentheses. Acronyms refer to the following institutions: STRI (Smithsonian Tropical Research Institute); LSUMNS (Louisiana State University Museum of Natural Science, Baton Rouge); ANSP (Academy of Natural Sciences, Philadelphia); FMNH (Field Museum of Natural History, Chicago); BMNH (Burke Museum of Natural History, University of Washington, Seattle).

Taxon	Collecting locality and voucher or tissue accession number(s)
Parula pitiayumi	Argentina: Tucumán-San Miguel de Tucumán (BMNH 54442, GAV649, JAG1752)
	Bolivia: La Paz Dept., Cerro Asunta Pata. (LSUMNS 22750)
	Bolivia: Santa Cruz Dept., Cordellera. (LSUMNS 18914, 19104)
	Bolivia: Santa Cruz Dept., Parque Nacional Noel Keonpff Mercado. (LSUMNS 18431, 18571)
	Costa Rica: Heredia Prov., Virgin del Socorro. (LSUMNS 16034)
	Panama: Darien Prov., Cana. (LSUMNS 2150)
	Panama: Veraguas Prov., Azuero Peninsula. (ANSP 5762, 7223)
	Trinidad: Chacachacare Island. (STRI CCPPI1, CCPPI2)
	USA: Louisiana, Cameron Parish. (LSUMNS 105302)
Parula americana	Jamaica: St. Elizabeth Parish. (STRI JAPAM1, JAPAM2)
Parula gutteralis	Panama: Chiriqui Prov., District Boquete. (LSUMNS 26458, 26459)
Parula superciliosa	Mexico: Guerrero St., Sierra de Atoyac. (FMNH 6142)
	Mexico: Michoacan St., Cerro de Tancitaro. (FMNH 5730)
Vermivora peregrina	Honduras: La Ceiba. (STRI HAVPE62)
Vermivora ruficapilla	USA: Washington, Yakima Co. (BMNH CSW5040)
Dendroica adelaidae	Barbuda: Martello Tower (STRI BUDAD1)
Dendroica tigrina	Jamaica: St. Elizabeth Parish (STRI JADTI1)
Coereba flaveola	Bahamas: Abaco. (STRI ABCFA2)

0.12 with eight rate categories (see Lovette and Bermingham 1999). In ATPase-based comparisons of our focal taxa *P. pitiayumi* and *P. americana,* pairwise divergences among haplotypes were very low and we estimated distances based on the uncorrected % transition + transversion divergence across all sites. We employed two phylogenetic methods—maximum-likelihood (ML) and maximum-parsimony (MP)—to reconstruct phylogenetic relationships among mtDNA haplotypes using Paup*. ML analyses were conducted using the quartet puzzling search algorithm of Strimmer and von Haeseler (1996), with parameters set as described above. Exhaustive MP searches on the subset of taxa from which we obtained long mtDNA sequences were run using only transversion substitutions owing to the rapid saturation of mitochondrial transitions at large genetic distances. We weighted all changes equally in our MP heuristic searches of trees best representing the relationships of the *P. pitiayumi* and *P. americana* ATPase haplotypes. In both MP analyses, bootstrap values were based on 1,000 heuristic replications.

Results and discussion.—Phylogenetic reconstructions based upon long mitochondrial sequences indicated that the four species currently placed in *Parula* constitute two pairs of closely related taxa, *P. gutteralis/P. superciliosa* and *P. pitiayumi/P. americana.* MtDNA sequence divergence between these two species pairs was high (9.4–10.0% HKY divergence). Rooted hypotheses of phylogenetic relationship (Fig. 1) that included representative *Vermivora* and *Dendroica* species suggested that those two *Parula* clades are not each others' closest relatives, with *gutteralis* and *superciliosa* being allied to *Vermivora* and *pitiayumi,* and *americana* allied to *Dendroica.* We tested that inference by comparing observed trees to trees identified in searches where the four *Parula* species were constrained to be monophyletic. In transversion parsimony searches, the observed tree (249 steps) was considerably shorter than the shortest constraint tree (276 steps). Similarly, a likelihood ratio test (Kishino and Hasegawa 1989) based on those alternative topologies demonstrated that the observed HKY ML tree (−Ln 10,597) was a highly significant improvement over the constraint tree (−Ln 10,705; $T = 5.49$; $P < 0.0001$). Those results indicate that the four currently recognized *Parula* species do not form a monophyletic group, at least in terms of their mitochondrial DNA gene tree. That pattern is consistent with previous suggestions that all or some members of the genus should be merged into the closely allied genera *Vermivora* or *Dendroica* (Eisenmann 1955, AOU 1998). On the basis of the present evidence, *gutteralis* and *superciliosa* appear to have affinities with *Vermivora,* and *pitiayumi* and *americana* with *Dendroica.* Evidence from additional, unlinked molecular loci are required to test that hypothesis, however, as hybrids between *P. americana* and several *Dendroica* species have been documented (Haller 1940, Cochrum 1952, Graves 1993) and the mtDNA gene tree (Fig. 1) could be incongruent with the corresponding organismal tree owing to past mtDNA introgression.

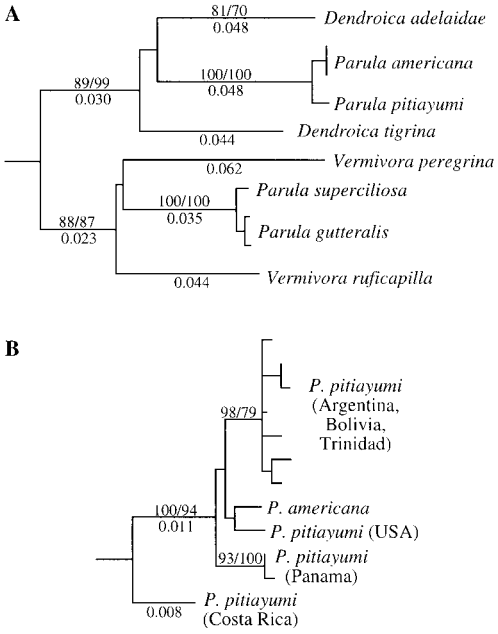

FIG. 1. (A) Maximum-likelihood tree depicting the phylogenetic relationships between the four *Parula* species and several representative *Dendroica* and *Vermivora* taxa. Reconstruction is based upon 3,639 nucleotides of protein-coding mtDNA sequence per individual and is rooted to sequences from *Coereba flaveola* (not shown). A transversion-based parsimony search identified an identical topology. (B) Maximum-likelihood tree depicting the phylogenetic relationships between *P. pitiayumi* and *P. americana* ATPase sequence haplotypes. In both trees, numbers below branches indicate HKY branch lengths >0.005; numbers above branches indicate ML reliability scores (left) and MP bootstrap proportions (right).

The *P. pitiayumi* and *P. americana* mitochondrial haplotypes were very similar and we included both taxa in our survey of geographic ATPase variation. The ATPase haplotype shared by the two *P. americana* individuals differed from the most similar *P. pitiayumi* haplotype by only six nucleotide substitutions (0.7% divergence), and that *P. americana* haplotype fell within a cluster of *P. pitiayumi* haplotypes in all reconstructions (Fig. 1). The mitochondrial evidence bolsters previous suggestions that *P. pitiayumi* and *P. americana* are conspecific (e.g. Hellmayr 1935, Paynter 1957, Lowery and Monroe 1968, Mayr and Short 1970, AOU 1983) by demonstrating that those taxa are not very different genetically and are not reciprocally monophyletic (Fig. 1B).

In situations such as this, where mitochondrial sequences show unexpectedly low divergence, it is particularly important to explore the possibility that the sequences represent slowly evolving Numts rather than the target mitochondrial sequences (e.g. Sorenson and Quinn 1998). Three lines of evidence suggest that the ATPase sequences reported here are mitochondrial. First, the pattern and magnitude of ATPase divergence was mirrored by the other three mitochondrial gene regions we sequenced from a subset of samples; those four gene regions together span more than 10 kb of the ~17 kb mitochondrial DNA genome and thus would represent an extraordinarily long nuclear translocation. Second, we found no unexpected nonsense or stop codons within coding regions, and levels of ATPase amino acid conservation between the *Parula* sequences were similar to those we have documented in other studies of closely related wood-warbler taxa (Lovette et al. 1998, 1999; Lovette and Bermingham 1999). Third, the sequences appear typically mitochondrial with a highly biased transition:transversion ratio (8.2) and a striking antiguanine bias at third positions (4.6%). We caution, however, that the second and third lines of evidence are considerably weaker than the first, because of the fact that translocated sequences maintain characteristic mitochondrial attributes for some time owing to the slower rate of molecular evolution in the nucleus (I. Lovette and E. Bermingham pers. obs.).

In our geographic survey of ATPase variation in *P. pitiayumi/americana,* we identified 12 unique haplotypes in our sample of 16 individuals. Only 36 nucleotide sites varied among those haplotypes and the 2 most divergent individuals differed by 22 nucleotide substitutions (2.6% uncorrected nucleotide divergence). We found little genetic divergence among haplotypes drawn from some geographically distant localities, particularly among South American samples (Fig. 1B). For example, haplotypes from Trinidad and Argentina differed at only two to four nucleotide sites (0.2 to 0.5% divergence) and we observed individuals with identical haplotypes in Bolivian and Argentinean collecting localities (Table 1) separated by 800 km.

In contrast to the near absence of mtDNA variation in South American *P. pitiayumi,* haplotypes from North and Central American locations showed modest differentiation. The largest differences (2.0 to 2.6%) among *P. pitiayumi/americana* haplotypes involved the single individual from Costa Rica, which was basal in the phylogenetic reconstructions (Fig. 1B). PCR or sequencing artifacts were unlikely to have produced the divergence seen in that individual, because we obtained an identical ATPase sequence from two separate rounds of extraction, amplification, and sequencing. Three individuals from eastern and western Panama had almost identical haplotypes that differed by 1.1–2.3% from those at other localities. Finally, the two samples of *P. americana* had identical haplotypes and formed a weakly differentiated clade along with an individual collected in Louisiana and identified by plumage traits

(S. Cardiff pers. comm.) as a vagrant *P. pitiayumi nigrilora,* the subspecies normally found in northeastern Mexico and southern Texas.

Although our geographic sampling of *P. pitiayumi* was coarse and a number of disjunct Central American and Mexican populations that could represent distinct evolutionary units were not sampled, the magnitude of mitochondrial variation among our widely spaced sampling locations was remarkably low. Considered together, *P. pitiayumi* and *P. americana* have a breeding range that extends from the boreal forests of northeastern Canada south through much of tropical Central and South America. The latitudinal breadth of that distribution, the phenotypic variation within *P. pitiayumi,* and the differences in migratory (AOU 1998) and song (Regelski and Moldenhauer 1996) behavior between some geographic populations suggested that those taxa would harbor considerable phylogeographic diversity, as has been found for other paruline warblers with both temperate- and tropical-zone breeding populations (Klein and Brown 1994, Milá et al. 2000). Nonetheless, the low magnitude of mitochondrial variation in *P. pitiayumi* and *P. americana* demonstrates that those taxa have experienced a recent genetic connection across vast geographic distances, either via expansion of an ancestral population or via gene flow among established populations. That pattern of low genetic variation contrasts markedly with the highly structured mitochondrial differentiation we have noted within many Neotropical *Basileuterus* warbler species (Lovette and Bermingham unpubl. data) and the large intraspecific genetic diversity documented within many other Neotropical resident birds. Although long-distance gene flow could result from migratory *P. americana* females remaining to breed with *P. pitiayumi* males south of *P. americana*'s normal breeding range, the close similarity of the Argentina, Bolivia, and Trinidad *P. pitiayumi* haplotypes and the lack of records of *P. americana* from continental South America (Ridgely and Tudor 1989: 494) argue against that scenario. An alternative and more likely possibility is that *P. pitiayumi* and *P. americana* have undergone an evolutionarily recent range expansion from a common ancestral population and that much of the phenotypic diversity that characterizes the present-day geographic populations we sampled is of relatively recent origin.

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Genetic Monogamy in Carolina Wrens (*Thryothorus ludovicianus*)

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Molecular comparisons have shown that socially monogamous passerines often have mixed reproductive strategies (Birkhead and Møller 1992, 1996). Pairs often cooperate in raising a brood, but each sex may pursue additional extrapair matings (e.g. Westneat 1987, Morton et al. 1990, Kempenaers et al. 1992). Further, females of some species lay eggs in nests of conspecifics (i.e. intraspecific brood parasitism, ISBP; reviewed in Hughes 1998).

Although considerable intra- and interspecific variation has been found in rates of extrapair paternity (EPP), causes for that variation remain unclear and additional data are warranted (Petrie and Kempenaers 1998). Further, few studies have been conducted on temperate-zone species that defend a territory and maintain a pair bond year round. In this study, we use multilocus DNA fingerprinting to examine paternity and intraspecific brood parasitism in Carolina Wrens (*Thryothorus ludovicianus*), a socially monogamous species that maintains a year-round pair bond and territory (Haggerty and Morton 1995). In addition, we report on breeding synchrony in Carolina Wrens because it is an ecological factor that may be related to paternity (Møller and Ninni 1998).

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Methods.—The study was conducted between March and August, 1996 and 1997, on a 43 ha mixed hardwood forest on the Tennessee Valley Authority reservation in Muscle Shoals, Colbert County, Alabama (34°49′N, 87°38′W). The overstory and understory are dominated by hackberry (*Celtis laevigata*) and privet (*Ligustrum vulgare*), respectively. During most of the breeding season, the ground-cover vegetation is dominated by honeysuckle (*Lonicera japonica*), poison ivy (*Rhus radicans*), and Virgina creeper (*Parthenocissus quinquefolia*).

Nest boxes were provided in late winter (5–6 per territory) and were readily used by Carolina Wrens (Haggerty and Morton 1995). Adults were captured near their nests with mist nets and approximately 30 to 100 μL of blood was collected from the brachial vein, stored in phosphate buffered saline/EDTA buffer (1996) or a lysis buffer (1997) and refrigerated. Blood samples from nestlings were collected in a similar way when they were 5 to 8 days old (hatching day = day 0). Adults were marked with a unique combination of colored leg bands and a U.S. Fish and Wildlife aluminum band. Parents caring for nestlings were the putative parents. Most adults had been previously banded as part of a long-term population study that began in 1988 (Haggerty and Morton 1995). Age (i.e. number of breeding years on study area) for adults that were fingerprinted ranged from 1 to 5 years ($\bar{x}$ = 1.6).

A 50 × 50 m grid system was established to help calculate size of the study area and to determine pair density. The 1996 and 1997 breeding-pair densities were 4.2 individuals/10 ha and 7.9/10 ha, respectively. A breeding-synchrony index was calculated for each year (Kempenaers 1993).

Multilocus DNA fingerprinting was conducted following the protocol of Loew and Fleischer (1996) using the Jeffreys 33.15 probe (Jeffreys et al. 1985). *Hae*III digested DNA of nestlings was usually placed in lanes between their putative parents for ease of scoring. Resulting autoradiographs were scored by counting number of fragments in a nestling's lane that were attributable to either or both parents profiles, and the number that were not (i.e. novel fragments). Pairwise band-sharing coefficients (*S*) were calculated according to Lynch (1991). A total of 84 offspring and 32 putative parents from 23 nests (i.e. a total of 116 individuals) were fingerprinted. Seven pairs were scored for two nests and nine pairs for only one nest. All offspring were fingerprinted for 16 of the 23 nests, but eight nestlings in seven nests were not analyzed because DNA was degraded or some other technical problem. Mean number of fragments per individual profile was 13.3 ± SD of 3.8 (range 6 to 24). Only fragments above about 3 kb were scored; hence, the smaller than normal number of fragments per profile. Typically, fragments below 3 kb were less variable than larger ones and they added little or no information to estimates of relatedness.

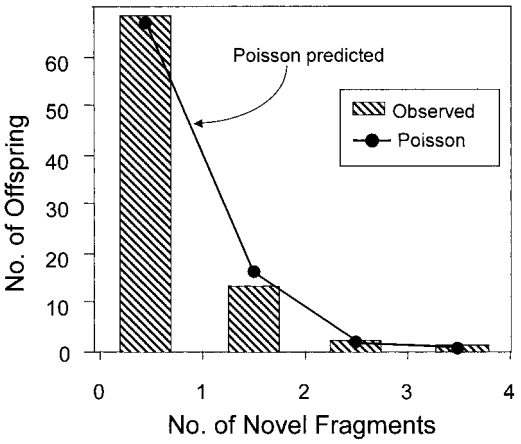

FIG. 1. Frequency of novel fragments among Carolina Wren offspring compared to their putative parents. Bars represent the observed frequencies. The line represents the theoretical distribution calculated from a Poisson distribution on the basis of mean number of fragments (0.238, n = 84) from nestlings with fewer than four novel fragments.

Results.—The synchrony indices for 1996 and 1997 were 17.7% ± 10 (n = 11 nests, 5 females) and 14.3% ± 11.0 (n = 39 nests, 19 females), respectively.

All DNA fragments found in offspring profiles were also found in the parent's profiles for 68 of the 84 offspring. For 13 offspring, we found one novel or nonattributable fragment. For two offspring, we found two novel bands and for one we found three. Mean number of novel fragments was 0.238 ± 0.55, corresponding to a mutation or artifact rate of 0.019 per fragment/generation. The distribution of novel fragments matches a Poisson expectation, on the basis of a mean of 0.238 (n = 84 profiles; Fig. 1). Because that match suggests that mutation alone can explain the extra fragments, we concluded, following the rationale of Westneat (1990), that there were no extra-pair fertilizations (EPFs) in our sample.

The mean value of *S* calculated for the 16 pairs of parents was 0.225 ± 0.13, which did not differ significantly from 11 random pairwise *S* values ($\bar{x}$ = 0.24 ± 0.1; t = 0.23, P = 0.82 [or Mann-Whitney U = 82.0, P = 0.77]). The predicted mean *S* for first-order relatives (R = 0.5) was 0.59 (equation 22 of Lynch 1991). Based on the level of background band-sharing, the probability of assigning parents incorrectly (ISBP) was 5.9×10^{-6}, whereas the probability of assigning the male parent incorrectly (EPF) was 1.9×10^{-4} (Bruford et al. 1998). The mean value of *S* for 84 comparisons of female parents and offspring was 0.52 ± 0.13, whereas *S* for male parents and offspring was 0.55 ± 0.11 (Fig. 2). The plot of *S* against

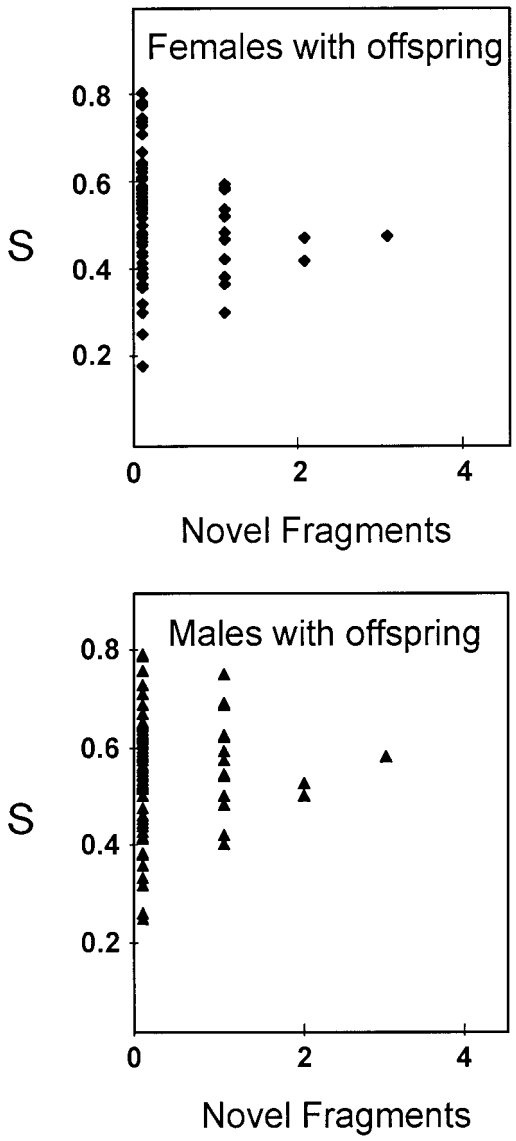

FIG. 2. Band-sharing among putative Carolina Wren parents and nestlings. Symbol location denotes the proportion of bands in a nestling's fingerprint shared with putative mother and father, and plotted against the number of bands in the nestling's fingerprint that were not in the putative parents fingerprint (novel fragments).

number of novel fragments shows that those individuals with 1, 2, or 3 novel fragments have high levels of band-sharing (Fig. 2), providing additional evidence that the 84 nestlings in 23 nests cannot be excluded from the adults attending those nests.

Discussion.—We found no evidence of a mixed reproductive strategy in our population of Carolina Wrens. The lack of ISBP was expected because we did not find any nests in which more than one egg had been laid over a 24 h period.

Factors that may affect opportunities for EPFs include breeding synchrony (Stutchbury and Neudorf 1998), population density (Westneat et al. 1990, Westneat and Sherman 1997, Møller and Ninni 1998), and mate guarding (Westneat et al. 1990, Currie et al. 1999). Westneat (1990) proposed that breeding synchrony should reduce frequency of EPP because males would be too busy guarding mates to engage in extrapair copulations (EPCs). Stutchbury and Morton (1995), however, proposed that breeding synchrony allows females to evaluate male quality and promotes EPP in some species. Our population had an overall low synchrony index value (i.e. 15.4% ± 11), which supports the Stutchbury and Morton (1995) hypothesis.

Although population densities during the years of this research were not the highest observed on the study site (i.e. 15 individuals/10 ha), territorial boundaries expand and are often shared even during low-density years (T. Haggerty pers. obs.). Therefore, we suspect that opportunities for EPFs existed and that a low density was not the primary cause for genetic monogamy in our population.

Although mate guarding may have occurred in our population, fledgling care should have limited the male's ability to guard their mates during the fertile periods of subsequent broods (Weatherhead and McRae 1990; but see Møller 1991, Conrad et al. 1998). Yet, we found no extrapair young in subsequent broods (i.e. 7 nests, 27 nestlings). Furthermore, territorial advertisement and defense in a visually occluded habitat should have made mate guarding difficult (Westneat et al. 1990) and some EPFs possible, yet none was recorded. Our population also has a low divorce rate (i.e. 2 of 36 cases from 27 pairs over 12 breeding seasons in which both pair members survived from one breeding season to the next), which is contrary to what is predicted when monogamy is enforced (Birkhead and Møller 1996, Gowaty 1996), but is expected when EPP rates are low (Cezilly and Nager 1995). Therefore, we doubt that mate guarding constrained females from engaging in extrapair activities.

As expected for a species with a low EPF rate (Birkhead and Møller 1996), males in our population contributed substantially to offspring care (Haggerty and Morton 1995, T. Haggerty unpubl. data). Although males do not incubate, they provide food to nestlings and females. In addition, nesting-interval data (Haggerty and Morton 1995) show that females lay and incubate new clutches well before fledglings from previous broods reach independence. Therefore, male care during the fledgling period may be essential if multiple broods are to be raised by a pair during a prolonged breeding season (Westneat et al. 1990). The threat of male desertion or reduced care

may constrain females from engaging in EPCs (Møller 1988, Burke et al. 1989, Dixon et al. 1994). Further, in sedentary species like the Carolina Wren, females may need mutually defended resources year round for their survival. Females engaging in EPCs may lose access to defended resources (Møller 1988, Westneat and Gray 1998). Most Carolina Wren mortality occurs during winter (Haggerty and Morton 1995, T. Haggerty unpubl. data) and females that have a mate may have a better chance of surviving and breeding another year than unfaithful and unmated females.

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Does Red-Cockaded Woodpecker Excavation of Resin Wells Increase Risk of Bark Beetle Infestation of Cavity Trees?

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The Red-cockaded Woodpecker (*Picoides borealis*) is unique among North American woodpeckers in that it nests and roosts nearly exclusively in living pines (*Pinus* spp.). Red-cockaded Woodpeckers make daily excavations at small wounds, termed ''resin wells,'' around their cavity entrance and on the bole of their cavity tree, from which resin flows down the tree (Ligon 1970). The woodpeckers also flake off loose bark which results in a smoother surface on the pine tree's bole. Those behaviors result in a resin barrier that serves as an effective defense against rat snakes (*Elaphe* spp.; Jackson 1974, Rudolph et al. 1990). Rat snakes regularly attempt to climb active Red-cockaded Woodpecker cavity trees (cavity trees currently in use for nesting and roosting) and are known to prey on Red-cockaded Woodpeckers when the resin barrier is inadequate (Jackson 1978b, Neal et al. 1993). The resin barrier is believed to increase the probability of a breeding pair's nest success and survival of roosting woodpeckers (Conner et al. 1998).

Red-cockaded Woodpecker cavity trees in eastern Texas, especially active cavity trees, are regularly attacked and killed by southern pine beetles (*Dendroctonus frontalis*) and occasionally by various species of engraver beetles (*Ips* spp.; Conner et al. 1991, Conner and Rudolph 1995, Rudolph and Conner 1995). The pine tree's resin, which woodpeckers use to create a barrier against rat snakes, serves also as the pine tree's primary defense against bark beetle infestation (Wahlenberg 1946, Hodges et al. 1977, Conner et al. 1998). The resin's flow rate and total production (yield) influence the pine tree's ability to physically repel a bark beetle attack. However, daily maintenance of resin wells by woodpeckers may decrease the pine tree's resin yield, and thus, reduce its ability to repel attacks by bark beetles.

We examined resin yield and bark beetle infestation rates in Red-cockaded Woodpecker cavity trees in longleaf (*Pinus palustris*), loblolly (*P. taeda*), and shortleaf (*P. echinata*) pines. Longleaf pine is widely known to produce greater yields of resin than loblolly and shortleaf pines and, as a result, is much more resistant to bark-beetle infestation (Hodges et al. 1977). Thus, if Red-cockaded Woodpeckers affect the ability of cavity trees to produce resin, the effect would most likely occur in loblolly and shortleaf pines. Also, if woodpecker activity at resin wells does increase susceptibility to bark beetles, the increase in bark-beetle-induced mortality should be

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greater in loblolly and shortleaf pines than in longleaf pines.

Methods.—We determined causes of mortality of Red-cockaded Woodpecker cavity trees on the Angelina National Forest (62,423 ha; 31°N15′N, 94°N15′W) in eastern Texas. The northern portion of the forest is predominantly covered by a mixture of loblolly and shortleaf pines on shrink–swell soils, whereas, longleaf pine is the dominant tree species in the deep sandy soils in the southern portion of the forest. Only a few remnant longleaf pines still occur on the northern portion of the Angelina National Forest. Small subpopulations of Red-cockaded Woodpeckers occur on both portions of the national forest (Conner and Rudolph 1989).

We visited all active and inactive (cavity trees previously used but currently not being used by woodpeckers) Red-cockaded Woodpecker cavity-tree clusters (a cluster is the aggregation of cavity trees used by a group of woodpeckers) during March through June from 1983 through 1998 to evaluate cavity tree status and condition. We used woodpecker activity at resin wells, amount of bark scaling, and condition of the cavity entrance as indicators of tree status (see Jackson 1977, 1978a). Active cavity tree clusters were visited several times per year. The age of many cavities within particular trees was determined by the year (and month if possible) they were completed, not the year that excavation began (see Conner et al. 1998). During each visit, we determined occurrence and causes of cavity tree mortality, such as wind throw, wind snap, fire, bark beetles, and lightning (see Conner et al. 1991). Cavity trees infested by bark beetles typically had numerous white ''popcorn-like'' pitch tubes of crystallized pine resin around wounds where individual attacking beetles had chewed through the bark and into the cambium of the pine tree's bole, or many small ''shotgun-pellet-like'' holes from which brood beetles had emerged. Dead cavity trees with signs of bark beetle infestation were examined closely to determine whether a lightning strike had contributed to the tree's death. Here we report observations for cavity trees that were infested and killed singly by bark beetles and not those killed during the growth of a beetle spot where multiple trees die in an expanding infestation. During such large infestations and epidemics, any pine tree in close proximity can be overwhelmed by the sheer numbers of bark beetles, regardless of the pine tree's ability to produce pine resin (Billings and Varner 1986). As a measure of beetle population levels, we obtained records of annual number of southern pine beetle infestations (beetle spots) and number of pines infested on both northern and southern portions of the Angelina National Forest in forest compartments where Red-cockaded Woodpeckers occur from the United States Forest Service Pest Management Office in Pineville, Louisiana (SPBIS, Southern Pine Beetle Information System data base).

During the growing seasons, we collected resin-yield data monthly from Red-cockaded Woodpecker cavity trees in loblolly–shortleaf pine habitat (1987 through 1988) and in longleaf pine habitat (1988 through 1989) (see Ross et al. 1995, 1997). We collected resin data from active and inactive cavity trees with naturally excavated cavities. We measured resin yield on sunny days by driving a 2.54 cm diameter circular arch punch (after Lorio et al. 1990) into the interface of xylem and phloem tissue on the pine tree's bole at approximately 1.4 m above ground. We punched holes on the south side of the bole between 0700 and 1000 h to minimize effects of diurnal variation in resin flow (Nebeker et al. 1988). We then placed triangular metal funnels directly under the wounds to channel exuded resin into clear plastic graduated tubes. Resin yield was recorded at 24 h after wounding to obtain a complete sample of the pine tree's preformed resin (see Ross et al. 1995, 1997). Only one sample per tree was taken per sampling period to avoid placing undue stress on active cavity trees. Because of the co-occurrence of loblolly and shortleaf pine cavity trees in woodpecker clusters on the clayey shrink–swell soils, as well as the similarity of those pine species in susceptibility to bark beetle infestation and magnitudes of resin production (Hodges et al. 1977), loblolly and shortleaf pine trees were considered as a single group for measurements of resin production and bark beetle mortality.

We used a paired *t*-test to evaluate the relative abilities of (1) longleaf pine cavity trees and (2) loblolly and shortleaf pine cavity trees to sustain resin production by comparing differences in spring resin yields of the same active cavity trees during subsequent years. Active cavity trees selected for that comparison contained completed, single cavities during the first year of comparison and remained active through the second year. Inactive cavity trees, used as controls, were measured during the same month and year. We also used Pearson correlation analyses to examine the relationship between spring resin yield from active cavity trees and the number of years the active cavity trees had been continuously used by Red-cockaded Woodpeckers. Only forest interior pines were used in those analyses because pines on the edges of forest stands are known to produce significantly more resin than pines in the forest interior (Ross et al. 1997). We also compared resin yield of active and inactive cavity trees within tree species throughout the growing season using a general linear model procedure (two-way factorial ANOVA, cavity tree status × month).

We totaled data over the 15 year study and used a chi-square test (adjusted for continuity) to examine differences in bark beetle infestation rates of cavity trees in longleaf versus loblolly and shortleaf pines, and to compare rates between active and inactive cavity trees within species groups. We also used a general linear model procedure (two-way factorial

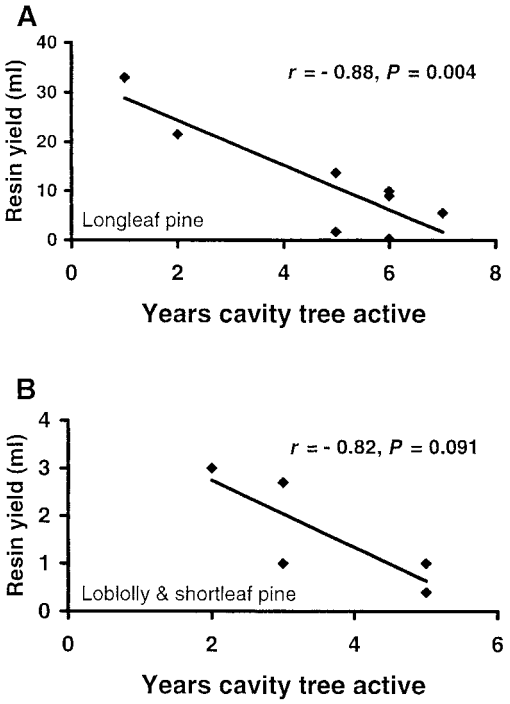

FIG. 1. Resin yield versus the number of years cavity trees have been actively used by Red-cockaded Woodpecker in longleaf (A) and loblolly and shortleaf pines (B) on the Angelina National Forest. Only data from forest interior cavity trees are used in these graphs, because pine trees on the edges of forest stands are known to produce greater resin yields than interior trees (Ross et al. 1997).

ANOVA) to examine differences in annual bark-beetle-induced cavity tree mortality rates among and within tree species throughout the 15 year study. All analyses were performed on SAS (release 6.12) for the PC (SAS Institute 1988).

Results.—Number of years that longleaf-pine cavity trees had been actively used by Red-cockaded Woodpeckers was negatively correlated with the pine tree's ability to produce spring resin ($r = -0.88$, $P = 0.004$; Fig. 1a). Although marginally significant, a similar relationship was observed in loblolly and shortleaf pines ($r = -0.82$, $P = 0.091$; Fig. 1b). Our comparisons of 24 h resin yield from cavity trees over a 1 year interval revealed that active loblolly and shortleaf pine cavity trees with single, completed cavities produced less spring resin in 1987 than they produced in 1988 (Table 1). During the same period, we detected no significant difference in spring resin yield from one year to the next among inactive loblolly and shortleaf pine cavity trees. We did not detect a significant difference in the yield of spring resin from active longleaf pine cavity trees in 1988

TABLE 1. Twenty-four-hour spring resin yield (mean ± SD) of active and inactive Red-cockaded Woodpecker cavity trees in longleaf and loblolly and shortleaf pines in eastern Texas between 1987 and 1989.

	Longleaf pine		Loblolly and shortleaf pines	
	Active ($n = 16$)	Inactive ($n = 28$)	Active ($n = 14$)	Inactive ($n = 28$)
	Spring resin yield (ml)			
1987	—	—	3.6 ± 1.6	5.3 ± 3.1
1988	10.1 ± 7.0	5.0 ± 3.7	2.2 ± 1.4	6.1 ± 5.3
1989	11.8 ± 10.9	4.4 ± 3.6	—	—
	Paired *t*-test[a]			
t	0.57	0.62	3.26	1.09
P	0.58	0.54	0.02	0.30

[a] Paired *t*-test results reflect differences between means within columns.

compared to spring resin yields from the same active cavity trees one year later (Table 1). Similar to inactive loblolly and shortleaf cavity trees, we detected no significant difference in spring resin yield from one year to the next among inactive longleaf pine cavity trees.

Two-way factorial ANOVA (cavity-tree status and month as factors) examining resin yield indicated that active longleaf pine cavity trees ($\bar{x} = 7.7$ mL resin, error df = 368) produced more resin than inactive longleaf-pine cavity trees ($\bar{x} = 5.4$ mL resin, $F = 15.29$, df = 1 and 7, $P = 0.0001$). We did not detect a difference in resin yield between active ($\bar{x} = 5.7$ mL resin, error df = 635) and inactive loblolly and shortleaf pine cavity trees ($\bar{x} = 6.6$ mL resin, $F = 3.32$, df = 1 and 8, $P = 0.07$). The interaction term in both ANOVAs was not significant ($F = 0.57$, $P = 0.7832$ and $F = 0.51$, $P = 0.85$, respectively).

A two-way factorial ANOVA (pine species and cavity-tree status as factors, df = 3 and 56) examining annual bark-beetle-induced mortality rates indicated that active cavity trees were killed at a higher rate than inactive cavity trees ($F = 15.99$, $P = 0.0002$) and loblolly and shortleaf pines were killed at a higher rate than longleaf pines ($F = 14.70$, $P = 0.0003$, Table 2). A significant interaction term ($F = 10.13$, $P = 0.0024$) indicated that the difference in mortality rates between active loblolly and shortleaf pines and active longleaf pines was greater than the difference between species for inactive cavity trees.

When standardized to deaths per 1,000 cavity-tree years, active loblolly and shortleaf pine cavity trees were killed by bark beetles at a rate of 81.8 per 1,000 cavity-tree years ($\chi^2 = 61.7$, $P < 0.001$), a 10.4-fold increase compared to the bark-beetle-induced mortality rate for inactive loblolly and shortleaf pine cavity trees (7.9 per 1,000 cavity-tree years, Table 2). Active longleaf pine cavity trees were killed at a rate of 10.4 per 1,000 cavity-tree years ($\chi^2 = 9.8$, $P = 0.002$),

TABLE 2. Bark-beetle-induced mortality of active and inactive loblolly, shortleaf, and longleaf pine Red-cockaded Woodpecker cavity trees in eastern Texas between 1983 and 1998.

Tree status and species	Cavity-tree years	Trees killed	Death rate per 1,000	Mean annual mortality rate %±SD
Active				
Loblolly and shortleaf pine	489	40	81.8	8.17 ± 7.0
Longleaf pine	772	8	10.4	1.06 ± 1.2
Inactive				
Loblolly and shortleaf pine	1,142	9	7.9	0.90 ± 1.4
Longleaf pine	2,757	5	1.8	0.24 ± 0.4

only a 5.7-fold increase relative to inactive longleaf pine cavity trees (1.8 per 1,000 cavity-tree years).

Bark-beetle induced-mortality rates differed between pine species. Active loblolly and shortleaf pine cavity trees were killed by bark beetles at 7.9 times the rate of active longleaf pine cavity trees, whereas inactive loblolly and shortleaf pine cavity trees were killed by bark beetles at 4.4 times the rate of inactive longleaf pine cavity trees. Although the difference is not statistically significant, it is important to note that active longleaf pine cavity trees were killed by bark beetles at 1.3 times the rate of inactive loblolly and shortleaf pine cavity trees ($\chi^2 = 0.322$, $P = 0.57$). Usually, longleaf pines are much more resistant to bark beetle infestation than loblolly and shortleaf pines (Hodges et al. 1977). Because of their greater vulnerability to bark beetle infestation, population levels of southern pine beetles were higher in loblolly shortleaf pine habitat ($\bar{x} = 97.0 \pm 82.6$ bark beetle spots) than in longleaf pine habitat ($\bar{x} = 16.2 \pm 20.2$) throughout the study ($t = 3.54$, df = 24, $P = 0.003$, see also Schaefer 1996).

Discussion.—We suggest that the observed higher rate of bark-beetle-induced mortality in active cavity trees is related to woodpecker excavation at resin wells. Regular, daily excavation at resin wells by Red-cockaded Woodpeckers may reduce the ability of active cavity trees to produce resin in response to beetle attack. Active Red-cockaded Woodpecker cavity trees were also more susceptible to bark-beetle-induced mortality than inactive cavity trees in all three species of pines (Conner and Rudolph 1995, Rudolph and Conner 1995, this study), which suggests that activity of woodpeckers at resin wells may increase the vulnerability of cavity trees to bark-beetle-induced mortality.

The rate of bark-beetle-induced mortality in active loblolly and shortleaf pine cavity trees was nearly 8 times greater than the rate of mortality in active longleaf pine cavity trees. When mortality rates were compared between active and inactive cavity trees within species groups, the increase in bark-beetle-induced mortality in loblolly and shortleaf pines was nearly double that in longleaf pines. That suggests that woodpecker activity on cavity trees is having a greater impact on susceptibility to bark beetles in loblolly and shortleaf pines than it is in longleaf pines.

Longleaf pines are known to produce larger amounts of resin than loblolly and shortleaf pines (Hodges et al. 1977), and are able to maintain a higher yield of resin when stressed by woodpecker excavation at resin wells than loblolly and shortleaf pines (Conner et al. 1998; Fig. 1). In spite of longleaf pine tree's known ability to produce higher yields of resin than loblolly and shortleaf pine trees, it appears that some active longleaf pine cavity trees still suffer bark-beetle-induced mortality. That may occur when longleaf pines are used continuously as cavity trees for 5 to 7+ years and their ability to produce resin drops to a point where they become vulnerable to bark beetles. Unfortunately, we do not have premortality resin data for the longleaf pines that were killed by bark beetles. The high resin production we observed in active longleaf pine cavity trees that we sampled relative to inactive cavity trees may represent the pine tree's response to repeated wounding by the woodpecker. In contrast, loblolly and shortleaf pines are known to generally produce less resin than longleaf pines. Because of their lower resin yields, when loblolly and shortleaf pines become active cavity trees, their ability to produce resin dwindles within the first year and they quickly incur an increased rate of bark-beetle-induced mortality.

The reduction in the ability of active cavity trees to produce sufficient resin—resin which serves as the pine trees' primary defense against bark beetles—appears to be a major factor affecting cavity tree mortality rates. When attacked by bark beetles, pine trees with a reduced capability to produce resin would be more vulnerable than pine trees with unimpaired resin production. The activity of Red-cockaded Woodpeckers at resin wells appears to reduce the cavity tree's resin production below what is necessary to ''pitch-out'' bark beetles, primarily in loblolly and shortleaf pines.

Daily excavation at resin wells coats Red-cockaded Woodpecker cavity trees with fresh pine resin, producing a constant ''wick'' of resin volatiles that evap-

orate and diffuse from trees. The presence of those resin volatiles around active cavity trees (volatiles that are known to be attractive to some bark beetles), may be a second factor explaining why bark-beetle-induced mortality is elevated in active cavity trees (see Payne and Coulson 1985, Coulson et al. 1995).

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Effects of Forest Harvesting on Nest Predation in Cavity-nesting Waterfowl

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Waterfowl populations in North America are threatened by habitat loss (Owen and Black 1990), but effects of habitat destruction and fragmentation on waterfowl nesting in forested landscapes are poorly known. Increased nest predation is often attributed to habitat fragmentation and may be particularly evident in smaller habitat patches and at habitat edges (Paton 1994, Andrén 1995). However, relatively few studies conducted in forest-dominated landscapes show edge effects at either natural or anthropogenic edges (Paton 1994, Andrén 1995, Pöysä et al. 1997). Lack of edge effects in forest-dominated landscapes may be due to relatively low predator species richness and abundance, and lack of predator attraction to edges (Andrén 1995). However, predator abundance and nest predation may increase with increased deforestation of the landscape (Andrén 1995, Hartley and Hunter 1998).

Effects of habitat destruction and fragmentation on nest predation of cavity-nesting waterfowl are unknown. We know of only one study of nest predation in cavity-nesting waterfowl in forest-dominated landscapes (Pöysä et al. 1997). This study found no edge effects at natural (lake) edges in a forested landscape, but did not investigate effects of forest harvesting. Thus, we experimentally investigated effects of forest harvesting on cavity-nesting waterfowl in the boreal mixedwood forest of western Canada, an important breeding and summering area for waterfowl. Although deforestation and fragmentation have proceeded relatively slowly in that region, large areas of forest have recently become available for harvesting. We used artificial waterfowl cavity nests to test the following hypotheses: (1) nest-predation levels in cutblocks (clearcuts with ≥8% of trees remaining) differ from predation levels in uncut forest, (2) nest-predation levels in riparian forest buffer strips differ from predation levels in uncut forest, (3) nest-predation levels in uncut forest vary with distance from the riparian forest edge, and (4) nest predation is higher around lakes in harvested versus unharvested landscapes.

Methods.—We conducted research from May through July in 1997 and 1998, in the boreal mixedwood forest surrounding 10 lakes in north-central Alberta, Canada. Six of the 10 study lakes were part of the TROLS (Terrestrial and Riparian Organisms, Lakes and Streams) project, a large-scale multidisciplinary study using experimental forest harvesting protocols at 12 lakes to determine effects of different buffer strip widths on aquatic and terrestrial boreal systems. Study lakes were in three clusters and ranged in size from 8.6 to 103.6 ha. Forests surrounding study lakes were dominated by trembling aspen (*Populus tremuloides*), balsam poplar (*P. balsamifera*), white spruce (*Picea glauca*), black spruce (*P. mariana*), and jack pine (*Pinus banksiana*).

Extensive commercial forest harvesting began in this region in 1993. Forest harvesting is carried out in two to three passes 10 years apart, creating a mosaic landscape of harvested patches of various ages and unharvested stands. Average cutblock size is approximately 30 ha and cutblocks contain ≥8% residual trees. When forest surrounding lakes is harvested, a forest buffer strip 100 m wide separates riparian vegetation and the adjacent lakeshore from harvesting activity. The purpose of buffer strips is to protect lake water quality. (Although riparian vegetation separated the forest from the lake edge around

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some study lakes, for clarity, we refer to the forest–riparian vegetation edge as the ''lakeshore'' hereafter.) Forest blocks around five study lakes were harvested once between September 1995 and April 1997 (harvested lakes). Harvesting removed about 10 to 40% of forest from the catchments of harvested lakes. Forest within 800 m of four of the five remaining study lakes was unharvested in both years of our study. The fifth lake was harvested to within 450 m in 1994. We refer to these five lakes as ''unharvested lakes'' hereafter. The amount of forest harvesting in catchments of unharvested lakes was 0 to 5%. Cavity-nesting waterfowl in the region include Bufflehead (*Bucephala albeola*), Common Goldeneye (*B. clangula*), Common Merganser (*Mergus merganser*), and Hooded Merganser (*Lophodytes cucullatus*). Potential mammalian predators of waterfowl cavity nests include short-tailed weasel (*Mustela erminea*), long-tailed weasel (*M. frenata*), mink (*M. vison*), marten (*Martes americana*), northern flying squirrel (*Glaucomys sabrinus*), and red squirrel (*Tamiasciurus hudsonicus*). Potential avian nest predators in the area include Common Raven (*Corvus corax*) and Gray Jay (*Perisoreus canadensis*), and although they are not true predators, Northern Flickers (*Colaptes auratus*) may also destroy eggs.

We placed four transects of artificial cavity nests around each lake. Each transect consisted of four nests approximately 30 m apart. Transects were ≥200 m apart. Around harvested lakes, we placed one nest transect in a cutblock at 50 m from the forest–cutblock edge, therefore approximately 150 m from the lakeshore, and one nest transect 50 m from the lakeshore edge of a 100 m wide forest buffer strip. We also located one nest transect in uncut forest 50 m from the lakeshore, and one nest transect in uncut forest 150 m from the lakeshore. At unharvested lakes, we placed two nest transects 50 m from the lakeshore and two nest transects 150 m from the lakeshore. Nest transects were in the same areas in both years of the study, although nests were not always on the same trees both years. Locating nest transects at both 50 and 150 m from the lakeshore allowed investigation of different levels of predation at different distances from the lakeshore.

We constructed artificial cavity nests with dimensions approximating the mean dimensions of natural Bufflehead and Common Goldeneye nest cavities (Bellrose 1980, Gauthier 1993, Eadie et al. 1995). Nests consisted of two 4.6 L plastic buckets wired together to create a cylindrical cavity 18 cm in diameter and 39 cm long with a 10 cm diameter entrance hole. We covered cavities with coarse, light brown burlap fabric to reduce conspicuousness and ensure predators could grip the plastic surface. We also placed a 3 to 5 cm wide strip of burlap inside the nest cavity and attached this to the lower edge of the entrance hole to allow predators to escape from cavities.

To facilitate predator identification in 1998, we attached hair-catchers at cavity entrances. Hair-catchers consisted of a flexible plastic strip ($0.16 \times 3 \times 29$ cm) fitted around the lip of the entrance hole and secured with double-sided indoor–outdoor carpet tape (Manco brand product 10-1). We stuck a strip of carpet tape (3×32 cm) on the surface of the plastic strip to collect hairs and feathers of potential predators entering artificial nests. We also placed a small piece of carpet tape at the top of the burlap tongue inside the nest boxes. Carpet tape remained strongly adhesive throughout the experiment.

We nailed nest cavities to trees approximately 2 m above ground, and placed leaf litter on the bottom of cavities. We placed one small wax-filled chicken egg (Pasitschniak-Arts and Messier 1995) and one plasticine egg of approximately the same size into each cavity. Small chicken eggs approximated the size of real Bufflehead and Common Goldeneye eggs (Gauthier 1993, Eadie et al. 1995). Before placing nests in cavities, we scented each egg with two to three drops of commercially produced duck scent to reduce bias against olfactory predators caused by the absence of adult birds and down nest lining from artificial nests (Willebrand and Marcström 1988, Pasitschniak-Arts and Messier 1995). We wore latex gloves when handling nest contents to reduce human odor (Pasitschniak-Arts and Messier 1995).

Nest-predation trials were 30 days long, paralleling Bufflehead and Common Goldeneye incubation periods (Gauthier 1993, Eadie et al. 1995). To help maintain olfactory stimuli at nests throughout the experiment, we added two to three drops of duck scent to each remaining egg or to the empty nest 13 to 16 days after starting trials. At that time in 1998 we also removed carpet tape with hair adhering to it before adding a new piece of tape. If there were no hairs on the tape we placed another layer on top of the first, to maintain consistent adhesiveness among nests.

At the conclusion of artificial nest trials, we examined eggs for predation and hair-catchers for hair samples in 1998. We considered a nest depredated if one or both eggs were pecked, bitten, broken, or removed. When possible, we identified mammalian nest predators by comparing tooth marks left in eggs to impressions made in plasticine using museum specimens. We could not identify avian predators to species level from egg damage. We removed hair samples from carpet tape using carbon tetrachloride (CCl_4). After removal, we cleaned hairs by soaking them in CCl_4 (Pasitschniak-Arts and Messier 1995) for 15 to 30 min. We measured hair length and diameter and determined stricture location, color pattern and shield appearance. We used confocal laser scanning microscopy to examine medulla structure and scanning electron microscopy to examine scale patterns. We identified hairs to species level by using identification keys based on those characteristics

TABLE 1. Predators identified at artificial nests located in riparian forest buffer strips (BS) and cutblocks (CB) adjacent to harvested lakes, and in uncut forest 50 and 150 m from the forest edge adjacent to harvested and unharvested lakes (H 50, H 150, and U 50, U 150, respectively).

Year	Predator	BS	CB	H 50	H 150	U 50	U 150
1997	Red squirrel	1	1		1	3	1
	Avian predator			1			
	Unidentified			3	4	4	2
1998	Red squirrel	3		3	1	5	8
	Red squirrel or northern flying squirrel					1	1
	Red squirrel or marten						1
	Short-tailed or long-tailed weasel	1					
	Avian predator					1	1
	Unidentified	2			3	5	3

(e.g. Adorjan and Kolenosky 1969, Wallis 1992) and samples taken from museum specimens.

We analyzed predation data in S-Plus 4.5 (MathSoft Inc. 1998) using generalized linear models (GLIM) with quasilikelihood functions. We used quasilikelihood functions in GLIM because those functions do not assume that errors conform to a particular distribution (McCullagh and Nelder 1989). We nested transects within lakes, and assumed that nests within transects were not always biologically and therefore statistically independent. We also included year (first or second summer of the study) in models, and examined interactions between transect type and year. We rejected null hypotheses at $P \leq 0.01$, rather than $P \leq 0.05$ because we conducted multiple comparisons with components of the data set (Miller 1981). We excluded nests destroyed by black bears (*Ursus americanus*) from analyses (54 in 1997, 56 in 1998). Black bears depredate nests of cavity-nesting waterfowl (Erskine 1972, Eadie et al. 1995), including those in nest boxes (J. E. Thompson pers. comm.). However, the artificial nest cavities we used were much more accessible and easier for black bears to destroy than natural cavities, and therefore did not provide a useful relative measure of black bear depredation of real cavity nests.

Results.—In 1997, we recorded 21 nest-predation events. Eleven of 48 nests (22.9%) were depredated at harvested lakes, compared to 10 of 58 nests (17.2%) at unharvested lakes (Table 1). Seven predation events were mammalian; tooth marks in eggs indicated that red squirrels were responsible for those. We identified one avian predation event based on egg damage. In the remaining 13 predation events, predators removed eggs and carried them away from nest sites; thus, we could not identify the predators.

In 1998 we recorded 39 nest-predation events. Thirteen of 38 nests (34.2%) were depredated at harvested lakes, compared to 26 of 66 nests (39.4%) at unharvested lakes (Table 1). Tooth marks in eggs demonstrated that red squirrels were responsible for 14 predation events. Analyses of hairs suggested that red squirrels were responsible for six additional predation events, in which eggs were removed from artificial nests. There were two avian predation events in 1998, identified by egg damage. Hair analyses suggested that two of the remaining 17 predation events were due to red squirrel or northern flying squirrel, one was due to short-tailed weasel or long-tailed weasel and one was due to red squirrel or marten. (Collection of multiple hair types at those nests, and difficulty distinguishing some hairs, precluded more precise identification.) Identities of predators in 13 events were unknown, due to egg removal and lack of hair samples.

Numbers of wax-filled chicken eggs versus plasticine eggs depredated did not differ significantly (1997: 15 wax-filled and 15 plasticine eggs taken; 1998: 39 wax-filled and 30 plasticine eggs taken; $G = 0.36$, $df = 1$, $P > 0.05$). Both eggs were removed from most nests (only plasticine egg removed: 7 nests; only wax-filled egg removed: 16 nests; both eggs removed: 38 nests).

At harvested lakes in both 1997 and 1998, nest predation was lower in cutblocks than on any other transect type. Only one nest in a cutblock was depredated (by a red squirrel) during our study. Nest predation was significantly lower in cutblocks than in uncut forest 150 m from the lakeshore; year did not significantly affect predation (Table 2, Fig. 1). (Extremely low predation in cutblocks precluded examination of an interaction between year and transect type using GLIMs.) Levels of nest predation in riparian buffer strips did not differ significantly from uncut forest 50 m from the lakeshore; again, effect of year was not significant, and there was no significant interaction between year and transect type, although standard errors were relatively large (Table 2, Fig. 1).

At both harvested and unharvested lakes, predation did not differ significantly in uncut forest at 50 versus 150 m from the lakeshore, and at harvested lakes, predation levels were not significantly differ-

TABLE 2. Results of generalized linear model analyses of predation at artificial cavity nests. Results significant at $P \leq 0.01$.

Null deviance	Residual deviance	Variables	SE	df	*F*	*P*
		Cutblocks vs. uncut forest 150 m from the lakeshore, at harvested lakes:				
49.59	38.69	Transect type	1.76	1, 46	10.55	0.002
		Year	0.42	1, 47	0.13	0.72
		Buffer strips vs. uncut forest 50 m from the lakeshore, at harvested lakes:				
49.08	33.47	Transect type	2.63	1, 34	1.30	0.26
		Year	2.29	1, 35	4.47	0.04
		Year × Transect type interaction	1.23	1, 32	2.74	0.11
		Uncut forest 50 m vs. 150 m from the lakeshore, at harvested lakes:				
51.73	50.25	Transect type	1.27	1, 35	0.18	0.67
		Year	1.29	1, 36	0.03	0.87
		Year × Transect type interaction	0.77	1, 33	0.05	0.82
		Uncut forest 50 m vs. 150 m from the lakeshore, at unharvested lakes:				
145.67	128.02	Transect type	2.21	1, 121	0.05	0.82
		Year	0.75	1, 122	9.87	0.002
		Year × Transect type interaction	0.49	1, 119	1.70	0.19
		Uncut forest 50 m from the lakeshore at harvested vs. unharvested lakes:				
92.46	86.25	Transect type	1.85	1, 70	1.83	0.18
		Year	1.21	1, 71	1.06	0.31
		Year × Transect type interaction	0.67	1, 68	0.22	0.64
		Uncut forest 150 m from the lakeshore at harvested vs. unharvested lakes:				
107.52	92.03	Transect type	1.75	1, 86	1.83	0.18
		Year	1.00	1, 87	6.08	0.02
		Year × Transect type interaction	0.60	1, 84	2.33	0.13
		Harvested lakes vs. unharvested lakes:				
247.51	230.59	Transect type	1.15	1, 207	0.13	0.72
		Year	0.55	1, 208	9.60	0.002
		Year × transect type interaction	0.35	1, 205	1.36	0.25

ent between years; the deviance values show that the model fitted the data very poorly (Table 2, Fig. 1). However, at unharvested lakes, there was a highly significant year effect (Table 2, Fig. 1); in 1998, predation increased 13% at transects 50 m from the lakeshore and 32% at transects 150 m from the lakeshore, compared to 1997. At both harvested and unharvested lakes, there was no significant interaction between year and transect type (Table 2).

Nest predation in uncut forest around harvested lakes, versus unharvested lakes, did not differ significantly at either 50 or 150 m from the lakeshore (Table 2, Fig. 1). Also, there were no significant year effects on nest predation at either distance from the lakeshore (Table 2, Fig. 1), and there were no significant year by transect type interactions (Table 2). Although not a statistically significant difference, in 1997 nest predation in uncut forest 150 m from the lakeshore was almost 40% higher at harvested lakes than unharvested lakes. In 1998, however, the difference was only 2%, due to increased predation at unharvested lakes.

When results for all nest transects were combined, lake treatment (harvested vs. unharvested) did not affect nest predation levels (Fig. 2); however, year did. Overall, predation was higher in 1998. The effect of year did not differ significantly between lake treatments (Table 2).

Discussion.—Almost all nest predators identified in our study were mammalian, and almost all mammalian predation events were unambiguously attributed to red squirrels. Pöysä et al. (1997) corroborate our findings by suggesting that waterfowl cavity nests are more frequently depredated by mammals than by birds. However, we may have underestimated the importance of avian predation if avian predators removed eggs from artificial cavity nests (Haskell 1995).

The number of plasticine and wax-filled chicken eggs attacked by predators did not differ signifi-

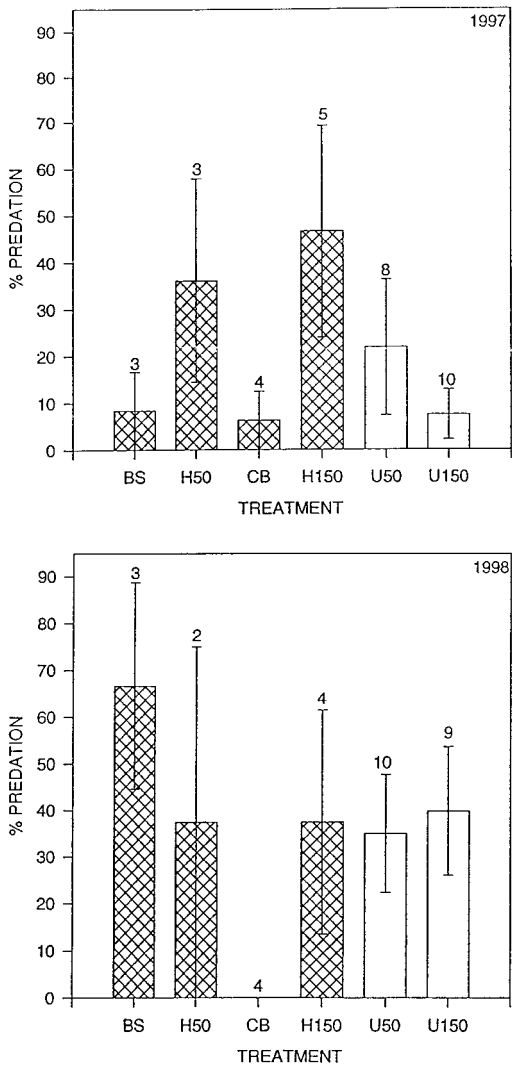

FIG. 1. Nest-predation levels (mean percent per transect ±1 SE) in 1997 and 1998 in riparian buffer strips abutting cutblocks (BS), 50 m from the lakeside forest edge at harvested lakes (H50), cutblocks (CB), 150 m from the lakeside forest edge at harvested lakes (H150), and 50 and 150 m from the lakeside forest edge at unharvested lakes (U50 and U150, respectively). Numbers above bars are number of transects included. Hatched bars = transects at harvested lakes, open bars = transects at unharvested lakes.

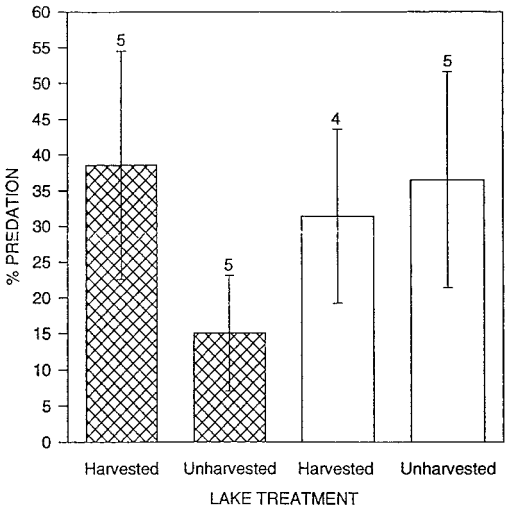

FIG. 2. Nest-predation levels (mean of percent predation per lake, for each lake type ±1 SE) in 1997 (hatched bars) and 1998 (open bars) at harvested and unharvested lakes. (Predation at all transects combined for each lake.) Numbers above bars are number of lakes included. (In 1998, all nests at one harvested lake were destroyed by bears, therefore four lakes were included in analyses.)

cantly. In most nests, both eggs were depredated, removing potential analytical problems associated with differential depredation of plasticine and real eggs in nest predation experiments (Bayne et al. 1997).

Artificial nest cavities were depredated significantly less in cutblocks than in comparable unharvested forest. Similarly, Ratti and Reese (1988) and Rudnicky and Hunter (1993) found predation of artificial ground and shrub nests was lower in clearcuts in a forest-dominated landscape. Red squirrels and martens tend to avoid clearcuts for at least six years after harvesting (Kirkland 1977, Snyder and Bissonette 1987, Thompson et al. 1989, Whitfield and Hall 1997). Because most of the mammalian nest predators in our study were red squirrels, lack of predation in cutblocks concurs with squirrel avoidance of harvested areas. Weasels do not exhibit clear responses to clearcutting, although data are sparse (Simms 1979, Thompson et al. 1989, Hansson 1994). Although there are few records of cavity-nesting waterfowl in clearcuts (R. G. Anderson and S. Woodley pers. comm.), if birds are able to nest in residual trees in cutblocks, they may experience lower nest predation and potentially higher nesting success for up to six years after forest harvesting.

Predation of artificial cavity nests in riparian forest buffer strips did not differ significantly from unharvested riparian forest. Red squirrel abundance in riparian forest buffer strips is not known to differ from unharvested forest (Whitfield and Hall 1997). However, Vander Haegen and DeGraaf (1996) found higher predation of open-cup nests in 20 to 80 m wide riparian buffer strips than in intact riparian sub-boreal Acadian forest. They identified red squirrels and

Blue Jays (*Cyanocitta cristata*) as important nest predators. In contrast, avian predators did not depredate nests in buffer strips in our study, unless they were removing eggs from nests, and thus could not be identified.

Paton (1994) concluded that nest predation was most likely to increase within 50 m of habitat edges, but studies in forest-dominated landscapes have failed to find edge effects (Andrén 1995). Similarly, predation on real and artificial waterfowl cavity nests is not known to increase in forests closer to lakeshores (Pöysä et al. 1997). Our study concurs with those conclusions. Nest transects in buffer strips were 50 m from both the lakeshore and cutblock edge, and predation levels in buffer strips did not differ from intact riparian forest. Predation on artificial cavity nests also did not differ in intact forest at 50 versus 150 m from the lakeshore, around either harvested or unharvested lakes. Mean nest-predation levels in uncut forest patches in our study were close to ranges found in artificial nests and real Common Goldeneye nests in nest boxes near lake shorelines in Sweden and Finland. Predation of cavity nests in these locales can range from 10 to 88% (Eriksson 1979, Fredga and Dow 1984, Pöysä et al. 1997).

Although negative edge effects due to clearcutting have not been demonstrated at smaller spatial scales (Andrén 1995), depredation of artificial ground nests can increase with increasing amounts of clearcutting at the landscape level (Hartley and Hunter 1998). However, our results for artificial cavity nests did not support that conclusion; predation levels did not differ around harvested and unharvested lakes. That may be due to the currently low level of forest harvesting in the landscape around our study sites.

Effect of year was significant in our study in analyses comparing nest predation at 50 versus 150 m from the lakeshore at unharvested lakes, reflecting the very low nest predation levels at 150 m from the lakeshore around unharvested lakes in 1997. Predation levels in buffer strips also differed greatly between years; however, the low number of nest transects and high variability in predation prevented that difference being statistically significant. Those patterns contributed to a significant year effect when we compared total predation at harvested versus unharvested lakes. Changes in nest predation between years of our study may be due to changes in the abundance and distribution of nest predators, for example the red squirrel, as a result of changes in squirrel food supply and weather conditions (Kemp and Keith 1970, Rusch and Reeder 1978, Gurnell 1983).

Our results show that 1 to 30 months after low-level forest harvesting, depredation of waterfowl artificial cavity nests did not increase, and predation was not higher at 50 m compared to 150 m from the forest edge adjacent to lakeshores. However, predation levels may change with increasing deforestation in the landscape, and increasing time since forest harvesting, especially if habitat changes induced by forest harvesting affect red squirrel abundance and distribution. The loss of nest cavities may negatively affect waterfowl more strongly than changes in nest predation due to harvesting, particularly at higher levels of forest harvesting. Also, when harvesting is extensive in the landscape, older trees, which are more likely to harbor nest cavities, may become concentrated into buffer strips adjacent to lakes. That may increase the risk of nest predation for cavity-nesting waterfowl by creating highly rewarding foraging patches for nest predators. Studies of real waterfowl cavity nests are required to determine the importance of those processes in the boreal forest of western North America.

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