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SPERM RELEASE IN MIGRATING WOOD-WARBLERS (PARULINAE) NESTING AT HIGHER LATITUDES

W. B. QUAY

Although there are many descriptions of behavioral and ecological differences and adaptations among species of wood-warblers (Parulinae) (e.g., Lawrence 1948, 1953; Ficken and Ficken 1965, 1967; Kammeraad 1966; Meanley 1969, 1972; Welsh 1971; Stewart 1973; Thompson 1977; Sealy 1979; Moore 1980), very little has been published concerning differences in their reproductive physiology and chronobiology.

I report here the results of a study on the timing of sperm release in spring migrant warblers while they are still south of their known breeding ranges. These results are considered significant because they demonstrate (1) a hitherto unreported adaptation in some warblers to short nesting seasons at higher latitudes, and (2) the successful application of the cloacal lavage technique (Quay 1984) for ascertaining the timing of spontaneous sperm release in free-living passerine birds.

METHODS

Study sites and times.—Two sites were used in the collection of data and cloacal lavage samples for study. The first was a residential yard at the NE end of the city of Galveston, Galveston Co., Texas (29°19′N, 94°48′W). This site was 1.6 km NW from the Gulf of Mexico and was known for passage of large numbers of warblers and other passerines during spring migrations. Birds were mist-netted at least several days per week throughout most of 1982. Migrant warblers were captured from 20 March to 13 May 1982, with the largest numbers in the last week of April and the first week of May. One hundred and nineteen warblers representing 25 species were captured and studied at this site.

The second site was a farm about 5 km NNW from the town of Foley, Lincoln Co., Missouri (39°08′N, 90°46′W). The site was located on the western bluffs of the Mississippi River Valley, and possessed a diversity of habitats that were sampled with mist-nets from 30 April to 7 May 1983, and 1 to 14 May 1984. One hundred and eighty-one warblers representing 18 species were captured and studied at this site.

Species groups.—The 300 individuals and 29 species used in this study were arranged latitudinally and categorized according to two groups. The first of these, designated "mid-latitude nesters," contains the 14 species whose nesting ranges either include the Foley site (species numbers 1–13) (Table 1) or are south of it (species 14) (Table 1). These species are listed in Table 1 in the order of the approximate latitude of the center of their geographic nesting range within the general north-south zone occupied by the two study sites. The boundaries and centers of nesting ranges were calculated chiefly from the maps in Peterson (1980) with finer detail in relation to the Galveston site calculated from data in Oberholser (1974). The second of the two artificial species groups, designated "highlatitude nesters," contains the 15 species whose nesting ranges are north of both of the study sites. The arrangement of these species in Table 2 was by the same criterion employed in Table 1. These arrangements of warbler species by latitude, and the designation of distances in relation

TABLE 1

NESTING DISTRIBUTIONS AND CLOACAL SPERM IN MIDLATITUDE-NESTING WARBLERS ARRANGED BY LATITUDINAL CENTER OF NESTING RANGE

Species	Number examined	Latitudinal center of breeding range	Distance from Galveston to center/southern edge of breeding range (km)	Proportions of birds with cloacal sperm					
				Galveston, Texas			Foley, Missouri		
				♂	♀	Unk.	♂	♀	Unk.
1. Yellow Warbler (*Dendroica petechia*)	2	47°	2010/770	0/1	0/1	0/0	0/0	0/0	0/0
2. Ovenbird (*Seiurus aurocapillus*)	26	44°	1620/560	0/1	0/0	0/8	0/2	0/1	1/14
3. Black-and-white Warbler (*Mniotilta varia*)	12	42°	1460/140	0/5	0/3	0/0	0/3	0/1	0/0
4. American Redstart (*Setophaga ruticilla*)	10	42°	1460/140	0/2	0/4	0/0	0/2	0/2	0/0
5. Blue-winged Warbler (*Vermivora pinus*)	1	41°	1430/710	0/0	0/1	0/0	0/0	0/0	0/0
6. Northern Parula (*Parula americana*)	1	40°	1360/60	0/1	0/0	0/0	0/0	0/0	0/0
7. Louisiana Waterthrush (*Seiurus motacilla*)	3	38°	960/160	0/0	0/0	0/3	0/0	0/0	0/0
8. Worm-eating Warbler (*Helmitheros vermivorus*)	3	37°	930/200	0/0	0/0	0/3	0/0	0/0	0/0

TABLE 1
CONTINUED

Species	Number examined	Latitudinal center of breeding range	Distance from Galveston to center/southern edge of breeding range (km)	Proportions of birds with cloacal sperm					
				Galveston, Texas			Foley, Missouri		
				♂	♀	Unk.	♂	♀	Unk.
9. Prothonotary Warbler (*Protonotaria citrea*)	11	37°	820/60	0/6	0/4	0/0	0/1	0/0	0/0
10. Kentucky Warbler (*Oporornis formosus*)	21	36°	750/170	0/1	0/6	0/1	8/10	0/2	0/1
11. Common Yellowthroat (*Geothlypis trichas*)	24	36°	Nesting range extends south of Galveston	0/6	0/4	0/0	6/9	0/5	0/0
12. Hooded Warbler (*Wilsonia citrina*)	7	36°	730/200	0/3	0/4	0/0	0/0	0/0	0/0
13. Yellow-breasted Chat (*Icteria virens*)	10	35°	Nesting range extends south of Galveston	0/0	0/0	0/3	3/5	0/0	0/2
14. Swainson's Warbler (*Limnothlypis swainsonii*)	1	34°	500/150	0/0	0/0	0/1	0/0	0/0	0/0
Totals	132			0/72			18/60		

TABLE 2

NESTING DISTRIBUTIONS AND CLOACAL SPERM IN HIGHLATITUDE-NESTING WARBLERS ARRANGED BY LATITUDINAL CENTER OF NESTING RANGE

Species	Number examined	Latitudinal center of breeding range	Distance from Foley site to center/southern edge of breeding range (km)	Proportions of birds with cloacal sperm: Galveston, Texas			Foley, Missouri		
				♂	♀	Unk.	♂	♀	Unk.
1. Blackpoll Warbler (*Dendroica striata*)	5	55°	1900/1490	0/2	0/0	0/0	0/2	1/1	0/0
2. Wilson's Warbler (*Wilsonia pusilla*)	2	53°	1580/1071	0/0	0/0	0/0	0/2	0/0	0/0
3. Tennessee Warbler (*Vermivora peregrina*)	23	53°	1520/890	0/9	0/9	0/0	2/4	0/1	0/0
4. Palm Warbler (*D. palmarum*)	19	52°	1440/800	0/0	0/0	0/0	0/4	0/0	5/15
5. Yellow-rumped Warbler (*D. coronata*)	74	52°	1430/780	0/0	0/1	0/0	0/37	0/15	0/21
6. Northern Waterthrush (*Seiurus noveboracensis*)	14	52°	1420/580	0/0	0/0	0/9	0/0	0/0	0/5
7. Magnolia Warbler (*D. magnolia*)	4	50°	1290/810	0/4	0/0	0/0	0/0	0/0	0/0
8. Black-throated Green Warbler (*D. virens*)	3	49°	1180/800	0/0	0/1	0/0	0/2	0/0	0/0

TABLE 2
CONTINUED

Species	Number examined	Latitudinal center of breeding range	Distance from Foley site to center/southern edge of breeding range (km)	Proportions of birds with cloacal sperm					
				Galveston, Texas			Foley, Missouri		
				♂	♀	Unk.	♂	♀	Unk.
9. Cape May Warbler (*D. tigrina*)	2	49°	1180/820	0/1	0/1	0/0	0/0	0/0	0/0
10. Bay-breasted Warbler (*D. castanea*)	5	49°	1150/940	0/5	0/0	0/0	0/0	0/0	0/0
11. Nashville Warbler (*V. ruficapilla*)	7	49°	1130/640	0/0	0/0	0/0	3/5	0/0	1/2
12. Canada Warbler (*W. canadensis*)	2	49°	1130/630	0/1	0/0	0/0	0/0	0/1	0/0
13. Blackburnian Warbler (*D. fusca*)	1	49°	1090/780	0/1	0/0	0/0	0/0	0/0	0/0
14. Mourning Warbler (*Oporornis philadelphia*)	4	48°	1000/400	0/1	0/0	0/0	0/3	0/0	0/0
15. Chestnut-sided Warbler (*D. pensylvanica*)	3	47°	880/550	0/1	0/0	0/1	0/1	0/0	0/0
Totals	168			0/47			12/121		

TABLE 3
CORRESPONDENCE OF CLOACAL SPERM INDEX (CSI) WITH SPERM NUMBERS PER MICROSCOPIC FIELD AND PER LAVAGE

CSI	Sperm/field	Sperm/lavage
0	0	0
1.0	0.00004	1–10
1.5	0.0125	1.5×10^3
2.0	0.5	6.0×10^4
2.5	2.0	2.4×10^5
3.0	5.0	6.0×10^5
3.5	12.5	1.5×10^6
4.0	33.0	4.0×10^6

to latitude, are approximations for comparative purposes only. The original data upon which they are based are fragmentary, and only parts of the total nesting ranges are involved in the calculations and the characterizations of nesting distributions.

Cloacal lavage specimens.—One or more (usually two) cloacal lavage specimens were taken from each bird within one hour of capture by mist-netting and prior to banding and release (Quay 1984). Disposable plastic pipette tips (Cat. No. OME 1–100, Orthomedics Inc., P.O. Box 247, Riverside, Connecticut 06878) were fitted with rubber bulbs and used to wash aliquots of about 0.05 ml distilled or deionized water in and out of the cloaca several times. The lavage (=wash) sample or specimen was then deposited on a clean 1 × 3 in. glass slide. This was immediately labeled with a diamond pencil, rapidly air-dried, and then stored in a dust-proof plastic slide box. Each pipette tip was discarded after a single use. The lavages were taken with minimal stimulation or trauma to the bird. Confirmatory evidence of this is provided by a male Kentucky Warbler that at the Foley site was first captured and lavaged in 1983 and was recaptured and relavaged three times in May 1984 without any signs of problems nor of notable reduction in sperm numbers per lavage.

Quantitative methods.—Quantitative evaluation of the dried cloacal lavages (CLs) was done on a Leitz Laborlux 12 microscope with phase contrast optics. The very refractile acrosomal region of the sperm, which glistened under phase contrast, was used as the feature upon which sperm counts were based. A "cloacal sperm index" (CSI) was determined from the average number of sperm per microscopic field (10× ocular, 40× objective lens). With the lavage area on each slide being of a standard size, an estimate of total number of sperm per lavage was made. The general accuracy and consistency of the CSI technique was checked by means of more detailed counts of sperm within microscopic fields at arbitrary intervals in a grid-like array over the entire lavage area (Table 3). The order of magnitude of numbers of sperm per lavage in males, as expressed by the CSI, is an indicator of level of sperm release. This is supported by results from several other kinds of studies on cloacal lavages of passerine birds (Quay, unpubl. data). Sperm release into the male cloaca in both resident and migrant warblers usually represents "spontaneous emission(s)" such as have been studied frequently in mammals (Orbach 1961, Lino et al. 1967, Fernández-Collazo et al. 1971) but rarely in birds (Reviers 1975; Quay, unpubl. data). Stimulated ejaculation, as in attempted as well as completed copulations, might also contribute to the sperm taken in avian cloacal lavages; however, I have found that male passerines isolated from females still have continuous emission of sperm into the cloaca. Unfortunately there is no technique available as yet for distinguishing cloacal sperm samples having this mode of release as compared with ejaculation during copulation. In females, however, all cloacal sperm under natural conditions must originate through inseminations by males.

RESULTS AND DISCUSSION

Recognition of sperm. —Recognition of sperm on slides containing dried CLs was a problem only when an excess of large particulate fecal material was present. Dilution with the lavage water and use of small-tipped pipette tips usually circumvented this problem. In nonstained and noncovered CLs sperm could be readily identified and photographed with phase-contrast microscopy (Fig. 1). Sperm can be distinguished easily from other fibrillar inclusions of the CLs by their unique morphology and refractile characteristics. Warbler sperm, like those of other examined passerines (Nicander 1970, Lake 1981), have a helical or spiraling sheath that extends over much of the length of the tail.

Sperm in midlatitude nesters. —Table 1 summarizes the results for cloacal sperm in midlatitude nesters, and gives the latitudinal characteristics of the species in this group. The four warbler species in which cloacal sperm were found were within their geographic nesting range, and individuals of these appeared to be actively territorial during the sampling period at the Foley study area. All sexually determinable (chiefly by plumage and cloacal regional characteristics) individuals having sperm were males; males outnumbered females at this time in the early phase of the breeding season. Midlatitude nesters not showing sperm at Foley in May were few. At Galveston, on the other hand, none of the midlatitude nesters showed cloacal sperm. The species represented here consisted of some that have nesting ranges with southern limits 60 to 770 km to the north (species 1–10, 12 and 14) (Table 1), and others that nest through Galveston's latitude but did not appear to have territories near the study site (species 11 and 13) (Table 1). Therefore, none of the migrant midlatitude warblers at Galveston had cloacal sperm, even though some of these were within a short distance of their known nesting ranges. This was most clear for species represented by larger samples containing males (Black-and-white and Prothonotary warblers and Common Yellow-throat) (Table 1).

Sperm in highlatitude nesters. —Table 2 summarizes the results for cloacal sperm in highlatitude nesters, and gives the latitudinal characteristics of the species in this group. None of these species showed sperm at Galveston at any time through the spring migration period, but four did at Foley. The southern or nearest limits of the nesting ranges of these four are about 640 to 1490 km to the north of Foley. These warblers, therefore, were certainly migrants far from their eventual nesting territories when they were releasing sperm into the cloaca. Most of the individuals were

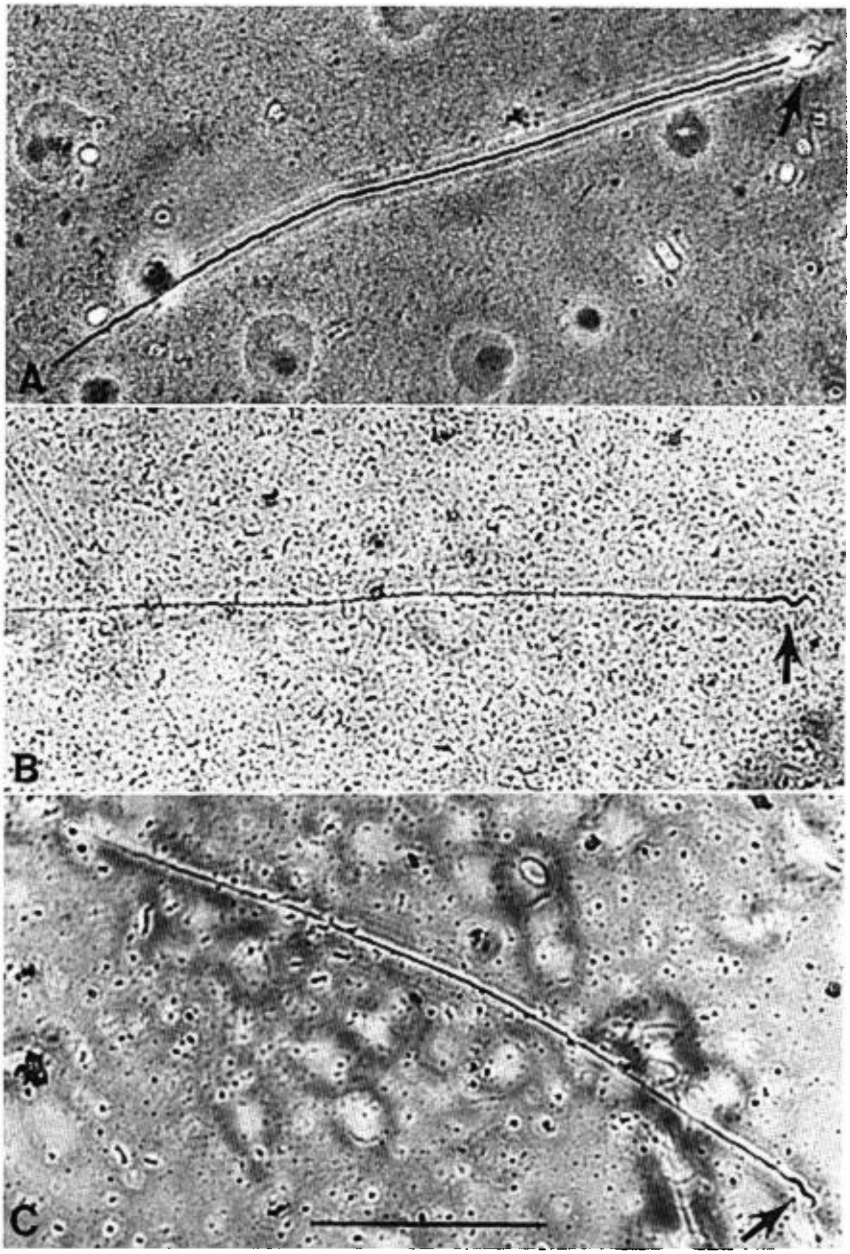

FIG. 1. Nonstained spermatozoa of spring migrant wood-warblers at Foley, Missouri, photographed with phase contrast microscopy. The head of each sperm is at the right

identifiable as males, or, lacking evident dimorphism externally, as sexual unknowns. The single Blackpoll Warbler having sperm was by external characteristics a female; however, it is probably premature at this time to accept this single occurrence as significant evidence of insemination prior to arrival at the nesting territory without either more definitive evidence of the bird's sex or additional examples from more samples. Nevertheless, Roberts (1936) has noted that "apparently mated pairs" of Blackpoll Warblers have been observed south of the known nesting range well into June.

It is significant that although Yellow-rumped (Myrtle) Warblers were the most numerous and most frequently sampled highlatitude migrant warblers at Foley, none had cloacal sperm. This is in marked contrast to the situation observed in Blackpoll, Tennessee, Palm, and Nashville warblers at the same site (Table 2). Thus, the data suggest that at least in several of the highlatitude nesters, and in none of the midlatitude nesters, there are species differences in the presence or absence of sperm release during spring migration (Tables 1 and 2). It is likely that precocious capacity for sperm release signifies an earlier physiological readiness for breeding. The actual occurrence of such breeding of course may depend upon ecological and behavioral factors as well.

Precocious physiological ability for breeding in spring migrants probably represents a component in adaptive strategies for reproductive success in the brief northern nesting season. Data in the literature on warbler reproduction, especially those concerning the timing of its phases and times of arrival and departure, allow some tentative suggestions about these adaptive strategies among warblers nesting at high latitudes. These birds are single-brooded in contrast with at least a few of the midlatitude nesters (e.g., Prothonotary and Swainson's warblers) that have been characterized as "sometimes" or "possibly" double-brooded (Harrison 1978). Additionally, the brevity of the northern nesting season is especially important for the Blackpoll Warbler, which is one of the last warblers to move to nesting grounds in the spring and which migrates from 4000 to 8000 km at that time (Bent 1953). As a group, the four northern species with cloacal sperm at Foley spend less time on their breeding/nesting range than does the Yellow-rumped Warbler (Table 4). In fact, similar available data for thirteen northern warbler species show that Blackpoll

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(arrows); the tail extends to the left. (A) Male Tennessee Warbler at 15:00, 11 May. (B) Sexually indeterminate Nashville Warbler at 19:00, 6 May. (C) "Female" (sexed by external characteristics) Blackpoll Warbler at 10:00, 13 May. All at same magnification; the bar scale is 20 μm long.

TABLE 4

TIMES SPENT ON THE BREEDING/NESTING RANGE BY HIGHLATITUDE SPECIES[a]

Species	Mean days from early spring arrival at breeding/nesting range to fall: Early arrival south of breeding/nesting range	Late departure from breeding/nesting range
Blackpoll Warbler	90	101
Tennessee Warbler	98	128
Nashville Warbler	108	168
Palm Warbler	113	162
Yellow-rumped Warbler	143	192

[a] Based on data from Bent (1953).

and Yellow-rumped warblers represent the two extremes in the range of times spent in the respective breeding/nesting areas.

Comparisons of the characteristics of breeding/nesting timing in northern warblers (Table 5) provides evidence of different adaptive strategies. In this table I have chosen six highlatitude-nesting species, the four releasing sperm at Foley, and the two not releasing sperm there but represented by the largest sample sizes for such species. Palm and Nashville warblers are the species whose arrival and early egg-laying dates are most compatible with the possibility of breeding before arrival at the nesting site (Table 5). There is, however, an unintentional bias in the "mean days from SRF" (sperm release at Foley), as other information suggests that passage through the Foley region by these species probably started before May and that my samples may therefore be skewed toward the later migration time. An analogous situation certainly affects the representations of timing by the Yellow-rumped Warblers in Table 5. On the other hand, a bias in the opposite direction may possibly occur in the representations of timing by Tennessee and Blackpoll warblers, as my samples may have missed late migrants or stragglers. In spite of these possible biases in my sampling, the differences between "earliest eggs" and "early arrival" dates support belief in a dichotomy between Palm and Nashville warblers on the one hand and Tennessee and Blackpoll warblers on the other, in their adaptive strategies. The differences between "earliest eggs" and "early arrival" are 8 to 9 days for the former and 21 to 22 for the latter (Table 5). The comparable differences are 8 and 37 days for Northern Waterthrush and Yellow-rumped Warbler, respectively (Table 5). The extent to which early physiological preparedness for breeding is part of the adaptive strategy for particular northern migrant species or populations can be determined only with the aid of additional kinds of studies.

TABLE 5

TIMING OF BREEDING/NESTING IN RELATION TO MIGRANT PRESENCE AND SPERM RELEASE AT FOLEY (SRF)

	Dates of		At breeding/nesting range[a]				
			Earliest eggs		Mean early arrival		Duration of nesting cycle (days)
Species	Sperm release	Species present	Mean days from SRF	Dates	Mean days from SRF	Dates	
Releasing sperm at Foley							
Palm Warbler	5–7 May	1–9 May	0	6 May	−9	27 April	24
Nashville Warbler	5–9 May	2–11 May	0	7 May	−8	29 April	22–23
Tennessee Warbler	7–11 May	7–13 May	23	1 June	2	11 May	Unk.
Blackpoll Warbler	13 May	4–14 May	28	10 June	6	19 May	21–22
Not releasing sperm at Foley			Mean days from Foley	Dates	Mean days from Foley	Dates	
Yellow-rumped Warbler	—	→9 May	21	26 May	−16	19 April	24–27
Northern Waterthrush	—	→9 May	19	25 May	11	17 May	Unk.

[a] Based on data from Bent (1953) and Harrison (1978).

The new findings in the present report open to view the hitherto unappreciated possibility that there are functionally important species differences in the timing of sperm release in relation to migration and the nominal start of the breeding/nesting season.

SUMMARY

Cloacal lavages (washes) were taken from 300 wood-warblers representing 29 species. Birds were captured at Galveston, Texas, and Foley, Missouri, in the course of spring banding studies. Phase-contrast microscopy was used to determine occurrence and numbers of spermatozoa on the lavage slides. Cloacal release of sperm at Foley occurred in four species of warblers that were within their geographic nesting ranges (Ovenbird, *Seiurus aurocapillus*; Kentucky Warbler, *Oporornis formosus*; Common Yellowthroat, *Geothlypis trichas*; and Yellow-breasted Chat, *Icteria virens*) and in four species that were still far south (>640 to 1490 km) of their nesting ranges (Tennessee, *Vermivora peregrina*; Nashville, *V. ruficapilla*; Palm, *Dendroica palmarum*; and Blackpoll, *D. striata*; warblers). None of the midlatitude-nesting species had cloacal sperm while passing through Galveston as spring migrants. The four highlatitude-nesting species with cloacal sperm at Foley spend less time on their northern breeding range than do Yellow-rumped Warblers (*D. coronata*), the most abundant non-sperm-releasing migrant warbler at Foley. Precocious sperm release during spring migration in some highlatitude-nesting species is probably an adaptation for more rapid onset of reproduction in areas with short summers and associated ecological limitations.

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