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Lack of sperm production and sperm storage by arctic-nesting shorebirds during spring migration

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Birds nesting at high latitudes may copulate during migration to arrive on the nesting grounds ready to breed. We surveyed 12 species of shorebirds during spring migration to determine whether (1) males produced abundant sperm and (2) females harboured functional sperm storage tubules (SSTs). Sperm production by males on migration was rare. Only four of seven species (9.8% of 41 males) of long-distance migrants harboured sperm, whereas all four species (100% of eight males) of short-distance migrants held sperm. In females, no long-distance migrants held sperm in their SSTs (n = 28 females) and SSTs were small compared to long-distance migrants collected on their breeding grounds. Our results indicate that shorebirds nesting at high latitudes were generally not reproductively active during migration and that any sexual behaviour on migration is unlikely to lead directly to fertilizations.

Birds nesting at high latitudes face a short breeding season. To cope with this constraint, northern migrants may copulate during migration to ensure against the possibility of not finding a suitable mate on the breeding grounds, to arrive on the nesting grounds ready to breed, or to allow a female to mate with a high-quality male (Moore & McDonald 1993). For such en route copulations to have a selective advantage to migrants nesting at high latitudes, two conditions must hold: (1) males must produce sufficient sperm during migration to ensure fertilization and (2) females must be capable of storing sperm in sperm storage tubules (SSTs) during migration and until the eggs are fertilized (Moore & McDonald 1993, Briskie 1996).

En route copulations have been observed in migrant songbirds (Quay 1985a, 1989, Moore & McDonald 1993), but it is unclear whether these copulations provide functional benefits. Quay (1985a) found that males of several migrant warblers (Parulidae) at spring stopover locations produced sperm and suggested that this process may somehow function to ensure breeding readiness. Further study revealed that female Tennessee Warblers *Vermivora peregrina* also contained sperm in their cloacae during spring migration (Quay 1989) indicating that copulations took place during migration. Quay (1989), however, did not examine the SSTs in female migrants and therefore was unable to determine whether en route copulations could lead to fertilizations. In a more recent study, Briskie (1996) found that the SSTs in four species of migrant warblers (including the Tennessee Warbler) were undeveloped and incapable of storing sperm. Although few individuals were examined, these observations suggested that en route copulations lack a functional significance and instead may be an epiphenomenon of gonadal enlargement and hormonal production during migration.

Despite the potential advantage of en route copulations, it is unknown whether they are widespread in migratory birds. To date, only passerine birds have been examined for either sperm production or sperm storage during migration (Quay 1985a, 1989, Briskie 1996, Jones & Norment 1998). In this study, we investigated whether en route copulations take place in 12 species of shorebirds (Charadriidae, Recurvirostridae, and Scolopacidae) by examining the reproductive tracts of individuals collected during spring migration to determine whether (1) migrant males produced sperm and (2) migrant females had

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functional SSTs. Migrant shorebirds provide an ideal study group for examining en route copulations because many species nest at high latitudes (Hayman *et al.* 1988), exhibit behaviours associated with reproductive activity at stopover locations (Piersma *et al.* 1991, Frodin *et al.* 1994), and may begin egg-laying shortly after arrival on the breeding grounds (Lanctot *et al.* 2000). Moreover, shorebirds also allow a comparison of sperm production and sperm storage during migration among several different mating systems and across differing degrees of sperm competition (Johnson & Briskie 1999).

METHODS

As part of a more comprehensive examination of migration ecology, shorebirds were collected during 6–18 May 1999 from playa wetlands located in Meade County, Kansas (37°18′N, 100°35′W) and 18–26 May 1999 at wetlands located on Quivira National Wildlife Refuge (NWR) in Stafford County, Kansas (38°10′N, 98°40′W). This period encompasses the peak of spring migration in this area (Thompson & Ely 1989). Twelve species were collected (Table 1), comprising nine 'long-distance' migrants and three 'short-distance' migrants. Long-distance migrants were defined as those species breeding exclusively in the arctic and subarctic regions of North America, a minimum of 2000 km from collecting sites (Hayman *et al.* 1988), and were the focus of our examination. Short-distance migrants were species known to breed locally (Thompson & Ely 1989), although some individuals may have been en route to locations further north.

As gonad samples were salvaged from birds collected primarily for other reasons, our sample sizes were dictated by other objectives. For most species we obtained samples from both males and females (Tables 1 and 2), except for Pectoral Sandpiper and Baird's Sandpiper, in which only females were collected, and for Western Sandpiper, in which a single male was collected (see Table 1 for scientific names of collected species). Although sample sizes were small for some species, we included all species for which we had a sample because of the paucity of data currently available on sperm production and sperm storage in migrant birds. All birds were collected under licence with the Kansas Department of Wildlife and Parks and the US Fish and Wildlife Service.

Within an hour of collection, reproductive tracts were removed and fixed in 10% formalin following Briskie (1996) and stored for 10–20 months before examination. In the laboratory, the length of the left testis of each male was measured to the nearest 0.1 mm with calipers. This provided a measure of testis size on migration, which was compared to values obtained from the literature for testis size on

Table 1. Frequency of sperm production and testis size in male migrant shorebirds. Sperm production is given by number of individuals with sperm in ductus deferens relative to number examined. Testis size presented as mean length in individuals collected in Kansas and as a percentage of testis size observed in birds collected on the breeding grounds.

Species	Proportion with sperm (%)	Mean length of testis (mm)	Relative testis size (%)
Short-distance migrants			
Killdeer (*Charadrius vociferus*)	1/1 (100)	8.4	105
American Avocet (*Recurvirostra americana*)	4/4 (100)	12.9	91
Wilson's Phalarope (*Phalaropus tricolor*)	3/3 (100)	6.8	76
Total	8/8 (100)		
Long-distance migrants			
Sanderling (*Calidris alba*)	1/4 (25)	6.1	51
Semipalmated Sandpiper (*C. pusilla*)	1/11 (9.1)	4.4	48
Western Sandpiper (*C. mauri*)	1/1 (100)	4.0	59
Least Sandpiper (*C. minutilla*)	0/2 (0)	3.2	53
White-rumped Sandpiper (*C. fuscicollis*)	0/15 (0)	4.6	45
Baird's Sandpiper (*C. bairdii*)[a]	–	–	–
Pectoral Sandpiper (*C. melanotos*)[a]	–	–	–
Stilt Sandpiper (*C. himantopus*)	1/5 (20)	7.1	70
Long-billed Dowitcher (*Limnodromus scolopaceus*)	0/3 (0)	6.5	67
Total	4/41 (9.8)		

[a] No males collected.

Table 2. Frequency of sperm storage by female shorebirds while on migration. Sperm storage given as numbers of females with sperm in sperm storage tubules (SSTs) relative to total number of females examined. Mean SST length estimated by measuring 30 SSTs per female. Range of mean SST lengths given when more than one female examined.

Species	Frequency of sperm (%)	Mean SST length (µm)[a]	
		Migration[b]	Breeding[c]
Short-distance migrants			
Killdeer	0/1 (0)	–	–
American Avocet	3/3 (100)	129.3 (1)	129.3–136.8 (2)
Wilson's Phalarope	1/8 (12.5)	40.2–68.3 (5)	110.3 (1)
Total	4/12 (33.3)		
Long-distance migrants			
Sanderling	0/2 (0)	24.1 (1)	–
Semipalmated Sandpiper	0/6 (0)	25.3–44.8 (5)	134.0 (1)
Western Sandpiper	–	–	–
Least Sandpiper	0/2 (0)	25.3 (1)	99.4–119.7 (2)
White-rumped Sandpiper	0/6 (0)	25.7–44.3 (4)	–
Baird's Sandpiper	0/3 (0)	23.0 (1)	104.2–125.6 (2)
Pectoral Sandpiper	0/1 (0)	–	96.3 (1)
Stilt Sandpiper	0/6 (0)	27.1–37.6 (4)	96.3 (1)
Long-billed Dowitcher	0/2 (0)	22.2–31.2 (2)	–
Total	0/28 (0)		

[a] Number of females given in parentheses. '–' indicates no data.

[b] SST length in females while on migration (includes all long-distance migrants) and for short-distance migrants without an egg in oviduct or large follicles in the ovary.

[c] SST length in females with egg in oviduct or large follicles in the ovary either in Kansas (short-distance migrants) or northern Canada (long-distance migrants).

the breeding grounds (i.e. Cartar 1985, Johnson & Briskie 1999, J.V. Briskie unpubl. data). The ductus deferens (the site of sperm storage in non-passerines) was then examined for the presence of sperm. Two sections (*c.* 1–3 mm) of the distal ends of the ductus deferens were removed (one section each from the left and right ductus deferens), placed in a drop of water on separate glass slides, and minced finely with forceps to empty the contents of their lumens. Slides were examined using a 400× phase contrast microscope. Sperm presence was determined by searching approximately 50 field-widths. Sperm were typically abundant when present (> 100 sperm per field) so this technique was unlikely to miss any individuals with functional sperm production. When only a few sperm were present on a slide (i.e. < 10 sperm per field), we made an exact count but when hundreds of sperm were present we simply classified them as 'abundant'.

In females, SSTs were dissected following the technique of Briskie and Birkhead (1993). Briefly, this involved removing three folds of the lamina propria at the junction between the uterus and vagina. Each fold was prepared as a wet mount, and each SST within each fold was examined for the presence of sperm under a 400× microscope. When SSTs were visible, 30 SSTs per female were measured (maximum length) and censused for sperm. To determine relative degree of SST development, two approaches were used. For long-distance migrants, we compared the SST size of individuals collected in Kansas to those of the same species collected on the breeding grounds near Churchill, Manitoba and Igloolik, Nunavut (J.V. Briskie and R. Montgomerie unpubl. data). Breeding birds were collected with eggs in their oviducts or with large follicles in the ovary and were dissected in the same way as the birds in this study. For short-distance migrants, individuals collected that lacked an egg in the oviduct or large follicles in the ovary were considered to be in migratory condition, whereas those individuals with eggs in their oviducts or with large follicles in the ovary were considered to be in breeding condition. SSTs from individuals of the same species in different conditions (i.e. migratory or breeding) were then compared to determine the relative degree of SST development.

Because small sample sizes precluded statistical analyses, we present our observations without use of inferential statistics and focus our interpretations on biologically relevant patterns. Conducting robust statistical analyses would require collecting many more birds than those used in this study, and considering the clear patterns we found, may not be ethical.

RESULTS

Sperm production by long-distance male migrants was rare. Sperm was found in only four of seven species of long-distance migrants, whereas males in all three species of short-distance migrants had sperm in their ductus deferentia (Table 1). Only 9.8% (*n* = 41 individuals) of individual long-distance migrants, but 100% (*n* = 8 individuals) of short-distance migrants held sperm in their ductus deferentia. Short-distance migrants held much more sperm (all > 500 sperm per field) than the individual long-distance migrants with sperm (all < 5 sperm per field).

Left testis length in long-distance migrants was typically about one-half to three-quarters the size of that found in breeding individuals (Table 1). In contrast, testis length of the three short-distance migrants was close to that expected for birds on or near their breeding grounds. In Stilt Sandpipers *Micropalama himantopus*, Sanderlings *Calidris alba* and Semipalmated Sandpipers *Calidris pusillus*, we had measures of testis size for both those individuals producing sperm and those that were not producing sperm while en route (Table 1). Left testis length was larger in individuals that produced sperm during migration than in conspecifics that did not produce sperm in the Stilt Sandpiper (8.0 mm, *n* = 1 vs. 6.4–7.6 mm, *n* = 3) and Semipalmated Sandpiper (5.6 mm, *n* = 1 vs. 3.2–4.9 mm, *n* = 8), but not in the Sanderling (5.7 mm, *n* = 1 vs. 5.6–6.6 mm, *n* = 3). Despite small sample sizes, this suggests that most birds in our sample were not producing sperm because their testes were still regressed.

Sperm storage was not observed in any long-distance migrant (Table 2); of 28 long-distance migrant females examined, none held any sperm. In contrast, sperm were observed in four out of 12 short-distance migrant females (Table 2). In 10 long-distance migrant females, SSTs could not be readily distinguished from the surrounding tissue of the mucosa, particularly towards the uterine end of the utero-vaginal junction (the usual location of SSTs). The oviduct was very small in all of the individuals without SSTs, and no sperm was visible anywhere in the vicinity in which the SSTs are located. Among the short-distance migrants, we also had difficulty distinguishing SSTs in two Wilson's Phalaropes *Phalaropus tricolor* and the single Killdeer *Charadrius vociferus* collected. Despite breeding locally, these particular individuals may have been migrants as well.

The size of SSTs was small in females collected on migration (Table 2). In those species for which we had information on females from their arctic breeding grounds (J.V. Briskie and R. Montgomerie unpubl. data), SST length was generally 2–4 times that of SSTs in females collected in Kansas (Table 2). This suggests that the lack of sperm storage by females while en route is due in part to the undeveloped state of their SSTs.

DISCUSSION

We found that shorebirds nesting at high latitudes were generally not reproductively active during migration. Most males in long-distance migrants lacked sperm in their ductus deferentia or produced sperm in such low numbers that fertilization was unlikely to be successful if these males had copulated during migration. This indicates that males do not undergo gonadal recrudescence during migration, at least not to the point at which sperm production becomes comparable to that observed in birds on the nesting grounds. In contrast, all short-distance migrants were found to have sperm present in the ductus deferens and in large numbers. While it is unclear whether these species were local breeders or were still migrating, that sperm were found in both short- and long-distance migrants indicates our investigative techniques were suitable for detecting sperm if it was present. Thus, the striking differences we found reflect meaningful biological patterns in sperm production between short- and long-distance migrant shorebirds.

Our observations of little or no sperm production by migrant shorebirds contrasts with that of migrant warblers, in which males of several species have been found to produce abundant sperm during migration (Quay 1985a, 1985b, 1986, Briskie 1996). Several hypotheses may explain the differences between these two groups of birds. First, most passerines tend to have shorter nesting cycles than shorebirds and so one would expect them to be less constrained in time than shorebirds if sperm production on migration were an adaptation to a short breeding season. That we found the opposite pattern suggests that time constraints might not be the explanation for sperm production by migrants. A second hypothesis relates to differences in extra-pair paternity between passerines and shorebirds. Recent studies using DNA profiling have revealed high levels of extra-pair paternity among many warbler species (e.g. Stutchbury *et al.* 1994, Yezerinac *et al.* 1995, Perreault *et al.* 1997), but relatively low levels in shorebirds (e.g. Heg *et al.* 1993, Pierce & Lifjeld 1998, Zharikov & Nol 2000). It is interesting to speculate that the seemingly more intense levels of sperm competition in warblers may be linked to their propensity to begin sperm production during migration. However, we found no sperm production in the polygynous White-rumped Sandpiper C*alidris fuscicollis* on migration, the only long-distance migrant shorebird with a non-monogamous social mating system in which we had a large sample (all other long-distance migrants were socially monogamous). Whether sperm competition plays a role in triggering early sperm production in other groups of birds will require a wider survey of migratory birds.

A third explanation for the difference between species with and without sperm production on migration is that the latter are constrained in using all available energy for migration. If the cost of developing testes and sperm production during migration are greater than any benefits received from copulating en route, then the energetics of transporting large testes may outweigh any advantages. This may be especially true for shorebirds nesting in arctic regions as they fly some of the longest distances of any migrant landbird. Although the extra weight of fully active testes are likely to be only 1–3% of total body mass, this may add considerably to the energetic costs of flight over long distances and thereby favour the development of the reproductive system only upon arrival on the breeding grounds (e.g. Piersma 1998).

We found no evidence that female long-distance migrants stored sperm while en route. Although SSTs were visible in many of the individuals we collected, no sperm were visible in any of them. SST length in migrating shorebird females was much smaller than that of females on the breeding grounds, suggesting that females do not fully develop their reproductive tract until after migration. A similar lack of SST development was found in Yellow-headed Blackbirds *Xanthocephalus xanthocephalus* and Pied Flycatchers *Ficedula hypoleuca* that had just arrived on their respective breeding grounds (Briskie 1994, Birkhead *et al.* 1997). In both of these species, SSTs were small upon arrival but then developed rapidly and began accumulating sperm within two weeks of being on the breeding grounds. Our results suggest a similar pattern may occur in shorebirds.

It is possible that the lack of sperm production in males and sperm storage by female shorebirds is due to our sampling location being too far south of the breeding grounds. The high frequency of sperm production found by Quay (1985a, 1985b, 1986, 1989) in passerines en route may be the result of his collection sites being closer to the breeding grounds than the birds collected in our study. Nevertheless, Briskie (1996) found no evidence of sperm storage in the warblers he studied at a site close to the breeding grounds. We have no data to test this idea for shorebirds, and it would be worthwhile to further study those species we classified as long-distance migrants at locations closer to their breeding grounds (e.g. on staging grounds in southern Canada). It should be noted that most of the long-distance migrants we collected in Kansas were already half-way or more between their wintering grounds in Central and South America and their breeding grounds in northern North America. If insemination during migration evolved as an adaptive reproductive strategy as proposed by Quay (1989) and Moore and McDonald (1993), then it might be expected that sperm production and sperm storage would be apparent by the time birds reached Kansas. This suggests that if reproductive tissues become functional during migration, it may not happen until birds are very close to their breeding grounds.

Our study of sperm storage in migrant shorebirds confirms earlier work on passerines that copulations en route are unlikely to lead to subsequent fertilizations and may best be explained as an epiphenomenon of gonadal enlargement and hormonal production during migration (Briskie 1996). Although mating behaviour on migration may still have an adaptive function, our examination of the reproductive anatomy of shorebirds suggests that the fertilization of eggs does not appear to be one of them. Whether this pattern holds for other groups of migratory birds remains to be determined. Functional sperm storage may occur in groups such as migrant waterfowl, in which pairing and copulations occur on the wintering grounds. However, for species which do not pair until after reaching the breeding grounds, the costs of copulating and storing sperm en route may outweigh any advantages accrued in advancing reproductive readiness.

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