Sperm size and sperm competition in birds

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SUMMARY

In a sample of 20 species of North American passerine birds we found no relation between sperm size and mating system like that previously reported in mammals (Gomendio & Roldan (Proc. R. Soc. Lond. B 243, 181 (1991)). Instead, we found a positive correlation between sperm length and the length of female sperm storage tubules (ssts) and a negative correlation between sperm length and the number of ssts. Both of these correlations suggest that the more than fivefold variation in sperm size we found among species can be explained by sperm competition for access to storage sites (ssts) in females. As longer sperm appear to be able to swim faster, selection should favour long sperm when ssts are in short supply; sperm long enough to fill an sst might also prevent access to ssts by the sperm of other males. Conversely, selection should favour shorter sperm when there is an advantage to sperm layering within an sst promoting a last-male mating advantage. Although we conclude that sperm competition influences sperm size in birds, little is known about the interactions between sperm and ssts. It seems clear, however, that detailed study of this interaction will provide a new dimension to the study of avian mating systems.

1. INTRODUCTION

Interspecific variation in the size of female gametes (ova) is extensive (see, for example, Kaplan & Saithe 1979), well documented (Heinroth 1922; Schönwetter 1960–1972), and, for the most part, easily explained. In birds, for example, variation in egg size varies over more than four orders of magnitude (Rahn et al. 1975), has been studied for more than 70 years (Huxley 1927), and is largely explained by variation in female body mass (Amadon 1943) and mode of offspring development (Nice 1962; Collins & Lecroy 1972; Sutherland & Rahn 1987).

In contrast, the factors influencing variation in the size of male gametes (sperm) are poorly understood. Studies of sperm structure (Retzius 1909) often reveal considerable variation in the size of sperm, even among closely related species (see, for example, McFarlane 1963), but the reasons for this variation have rarely been examined. To the best of our knowledge, the only quantitative analyses of sperm size variation among a wide variety of species are two recent studies of mammals (Cummins & Woodall 1985; Gomendio & Roldan 1991) and one of insects (Dybas & Dybas 1981). Based on data from 264 species in 149 genera, Cummins & Woodall (1985) found a general negative relation between total sperm length and male body mass in mammals, but this relation was significant in only three of six orders examined, and in one of these (Chiroptera) it was significantly positive. Recently, Gomendio & Roldan (1991) reported a positive correlation between maximum sperm velocity and sperm length across five diverse mammal species, and suggested that sperm competition for access to unfertilized ova would thus select for longer sperm. Within primates and murid rodents, they found that sperm was longer in polyandrous than in monandrous species, thereby supporting their hypothesis that longer sperm are favoured by selection when sperm competition is most intense (i.e. when females mate promiscuously).

In a study of eight species of minute featherwing beetles (Coleoptera: Ptiliidae) in Sri Lanka, Dybas & Dybas (1981) discovered a strong correlation between sperm length and the lengths of both the spermathecal lumen and the spermathecal duct in females. As sperm are particularly large in this family (sometimes being longer than the male), and only a small number can be accommodated in the female (28 in Bambaria invisiblis), they also suggested that sperm competition might have resulted in morphological coadaptation between the sizes of male sperm and female spermathecae. Such coadaptation would allow the first male to mate with a female to completely fill her spermatheca and thereby exclude any potential competitors for access to the single large egg. The remaining literature on sperm size variation in animals is largely descriptive.

In this paper we present a detailed analysis of sperm length variation in 20 species of North American passerine birds. By using data on morphology, mating systems and copulation behaviour, we are able to reject a number of hypotheses that might have explained the more than fivefold interspecific variation in sperm length in the species that we studied. Most important, our analyses suggest that sperm competition might account for much of the variation and provide a
foundation for understanding the enormous amount of variability in the size of this gamete among species.

2. METHODS

We examined sperm taken from 45 male birds of 20 species (in 17 genera and seven families; see Figure 1) during their breeding seasons in Ontario, Manitoba and the Northwest Territories, Canada in 1988–1990. We collected sperm either by gently massaging the cloacal protuberance of live birds or by salvaging sperm from the seminal glomeru of freshly killed specimens (see Wolfson 1952). Semen samples collected in this manner were smeared on a clean glass slide, allowed to air dry, and then examined as unstained mounts at 400× magnification under a phase contrast microscope. Whenever possible, ten haphazardly chosen sperm from each individual were measured using an ocular micrometer. For each sperm we measured the total length as well as the lengths of the ‘head’ (acrosome plus nucleus plus midpiece) and ‘tail’ (principal piece). Means were calculated for each species by averaging the mean values per individual.

We looked for relations between sperm length and a variety of other variables that we expected might be related to sperm length. Body masses for both sexes were taken from Dunning (1984). Mating systems were either determined by us in the field (seven species), by searching the literature (four species), or by communication with other workers (nine species). When the mating system of a species was known to vary geographically, we used the predominant mating system at or near the site where we collected our samples. Male testis lengths were taken from more than 25 museum specimens of each species collected during the breeding season at or near our study sites. Because testis size varies during the breeding season, we fitted a second-order polynomial to the data from each species and used the maximum from each of these regressions to represent the peak testis length during breeding. We obtained data on egg dimensions from Harrison (1979), and used Hoyt’s (1979) formula to estimate egg volume.

Female birds store sperm in specialized sperm storage tubules (srt) lining the oviduct at the uterovaginal junction (see, for example, Howarth 1974; Shugart 1988). We reasoned that srt morphology might influence sperm length because some srt store sperm for six days to ten weeks, depending on the species (review in Birkhead 1988), and sperm longer than the srt would be removed during egglaying (see Discussion). To obtain data on the size and number of srt in females of each of the species for which we had sperm sizes, we collected, preserved and examined oviducts from a total of 59 females taken just before or during their egg-laying period. The reproductive tract of each female was preserved in 10% (by volume) buffered formalin and later dissected and examined as a wet mount at 100× with a phase contrast microscope (see Briskie (1990) for details). Oviduct length was measured using a thread placed along its uncoiled length from the infundibulum (the site of fertilization) to the cloaca. Some srt are branched, but because sperm were stored primarily in the distal ends of these srt, each branch was counted as a separate sperm storage site. All of the srt occurring on three haphazardly chosen oviductal folds in the
uterovaginal region were counted, and the mean value per fold was multiplied by the number of folds to get a total number of srs per female. sperm length (i.e. inside length) was measured on a haphazardly chosen sample of five clearly visible srs from each of these three folds for a total of 15 srs per female. As for males, mean values of each variable were calculated for each species studied and only these means were used in further analyses.

In all comparative analyses, we used methods described by Harvey & Pagel (1991) to control for phylogeny. Such methods are required because traits may be similar in closely related species simply because of common ancestry rather than independent evolution, and a failure to control for this biases both sample sizes and the influence of some traits on the analysis. First, we used Sibley & Ahlquist (1990) to construct a phylogeny for the species studied (figure 1). Then, using the Evolutionary Covariance Regression (ECR) program (M. Pagel, unpublished software), we calculated unique linear comparisons or 'contrasts' (Grandage 1988) for each node in the phylogeny at which there was variation in the independent (or 'test') variable (see Harvey & Pagel 1991). Thus sample sizes in these analyses are the number of independent contrasts and not the number of species used in the analysis.

A variety of statistical methods can be used to assess the relations between linear contrasts (see Harvey & Pagel 1991). We used simple and multiple regression analyses because the data satisfied the appropriate statistical assumptions: rank correlation or binomial tests on the contrasts provided identical results. In all cases we forced the regression through the origin as recommended by Harvey & Pagel (1991).

3. RESULTS

Sperm length varied little within the 20 species studied but varied more than fivefold across species (figure 2). Variation in sperm length across species resulted largely from variation in the length of the tail (i.e. principal piece) and not to variation in the length of the head (i.e. acrosome, nucleus and midpiece; figure 3). Nested ANOVA revealed that 99.4% of the variation in sperm length occurred among species.

![Figure 2. Relation between sperm length and body mass for 20 species of passerine birds from four different mating systems: monogamous (filled circles), polygynous (open diamonds), polygynandrous (open triangles), and serial polyandry (open squares). Numbers refer to species listed in figure 1.](image)

![Figure 3. Relation between total sperm length and sperm head length (open circles; r = 0.38, p = 0.13) or tail length (filled circles; r = 0.998, p = 0.0001) for 17 of the passerine species studied.](image)

Table 1. Relation between sperm length and both male body mass and various female traits related to sperm storage and fertilization

<table>
<thead>
<tr>
<th>Variable</th>
<th>r</th>
<th>F</th>
<th>p</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>male body mass/g</td>
<td>-0.38</td>
<td>2.57</td>
<td>0.13</td>
<td>16</td>
</tr>
<tr>
<td>testis length/mm</td>
<td>0.39</td>
<td>2.48</td>
<td>0.14</td>
<td>16</td>
</tr>
<tr>
<td>egg volume/mm³</td>
<td>-0.38</td>
<td>2.24</td>
<td>0.16</td>
<td>15</td>
</tr>
<tr>
<td>oviduct length/mm</td>
<td>-0.09</td>
<td>0.13</td>
<td>0.73</td>
<td>16</td>
</tr>
<tr>
<td>number of srs</td>
<td>-0.82</td>
<td>28.2</td>
<td>0.0001</td>
<td>16</td>
</tr>
<tr>
<td>sperm length/µm</td>
<td>0.94</td>
<td>100.1</td>
<td>&lt;0.0001</td>
<td>16</td>
</tr>
</tbody>
</table>

correlate with mating system in a variety of animal species (Harcourt et al. 1981; Cartar 1985; Kenagy & Trombulak 1986) and may, indeed, be a better index of the intensity of sperm competition experienced by males than simple mating system categories based on field observations (Møller 1988, 1991). However, testis length as an index of mating system was not significantly correlated with sperm length across species (table 1). As we had only seven species in our sample that were not classified as monogamous, and many of those were closely related (figure 1), our sample of independent evolutionary events is too small to permit statistical analysis of sperm length against mating system as a categorical variable. It is worth noting, however, that the average sperm length in polygynous and polygynandrous species (114.9 μm, n = 4 species), where sperm competition is thought to be most intense, is smaller than that in monogamous species (126.9 μm, n = 13). These two samples should not be compared statistically because body mass and phylogeny have not been controlled. The trend, however, is in the opposite direction to that found in both murid rodents and primates, again not controlling for body mass and phylogeny (figure 2, a and b, respectively, in Gomendio & Roldan 1991). Moreover, the range of sperm lengths in monogamous birds is at least as great as that in polygamous species (figure 2). We conclude, therefore, that there is no evidence for a relation between sperm length and mating system in passerine birds.

Next we looked for correlations between sperm size and various female traits. Because sperm size (in particular, tail length) appears to influence maximum swimming velocity (Gomendio & Roldan 1991), we examined the correlation between sperm size and oviduct length (with female body size controlled statistically), reasoning that faster sperm would gain a particular advantage when swimming longer distances. There was, however, no significant partial correlation between sperm length and oviduct length across species (table 1).

We also examined the relation between sperm size and egg size because larger eggs are likely to have thicker vestsments, and longer sperm can generate greater force (Katz & Drobnis 1990) to penetrate ova at fertilization. Ideally, sperm size should be compared with the size of the ovum at fertilization, but data on ovum sizes are not available for the species that we studied. Egg size is known to be highly correlated with ovum size across species (Ar & Yom-Tov 1978; Sutherland & Rahn 1987), however, the partial correlations between sperm and egg size was not significant (table 1). This lack of correlation also rules out the possibility that longer sperm may simply result from a correlated response of sperm size to selection on the size of female gametes.

As sperm storage tubules (ssts) are thought to be important in sperm competition (Birkhead 1988), we examined the relation between sperm size and both the number and length of ssts in females (controlling for female body mass in each case). The partial correlations with sperm length are significant for each of these variables (table 1): positive with the length (figure 4a, c) and negative with the number (figure 4b, d) of female ssts. The correlation between sperm length and sst length is relatively strong despite the fact that a single sst can accommodate from one to three layers of sperm stacked end to end, depending upon the species (J. V. Briskie & R. Montgomerie, unpublished data). ssts in all bird species that we studied could store at least one layer of sperm, but the relation between sperm and sst length across species (figure 4e) shows that females in half of these species could store two or more layers of sperm in their ssts.

4. DISCUSSION

Both the positive correlation between sperm length and sst length and the negative correlation between sperm length and the number of ssts suggest that sperm competition for access to ssts may account for much of the interspecific variation in sperm length in birds. Because average sst length was longer than average sperm length in all of the species that we studied (figure 4a), it is clear that sst length does not simply evolve to be long enough to accommodate male sperm. The apparent absence of a relation between sperm length and mating systems in birds further suggests that the processes of sperm competition occurring at the gametic level may differ from those expected from observations of the social aspects of avian mating systems.

Opposing selection pressures might be expected to act on sperm length in birds for several reasons. First, when swimming speed increases with sperm length (Gomendio & Roldan 1991), selection should favour an increase in sperm length whenever there is competition between sperm from different males for access to unfertilized ova or ssts. More work is needed to confirm that longer sperm swim faster because the only support for this hypothesis comes from an analysis of data from five mammal species (Gomendio & Roldan 1991), and there appears to be no reason to expect such a relation based on the hydrodynamics of swimming sperm (Alexander 1982). Second, whenever sperm are stored before fertilization, selection should favour sperm shorter than or equal to the length of the storage site: sperm that cannot fit entirely into an sst are likely to be swept out of the urogenital tract whenever an egg is laid (Lorenz 1966). However, sperm that completely fill an sst could exclude other sperm from entering and thereby prevent the safe storage of sperm from potential competitors. Thus a correlation between the lengths of sperm and ssts (e.g. figure 4a, c) might be expected across species because of competition among males to fill ssts with sperm. Third, as suggested by Parker (1982), there ought to be a trade-off between the size and number of sperm produced by a male, everything else being equal. Our data, however, are not consistent with this hypothesis. Because, in birds, there is a positive correlation between testis size and the number of sperm (both controlled for body mass) in an ejaculate (Møller 1988), Parker’s (1982) hypothesis would predict a negative correlation between relative testis size and sperm length. In the birds that we studied, however, the correlation was positive, although not statistically significant (table 1).

Although the correlation between sperm length and sst length may be a simple consequence of the processes influencing male–male competition for access to safe storage sites, this cannot explain why sperm and ssts vary so greatly in size even among closely related species of similar body mass (figure 2). We suggest that this interspecific variation in sperm length may result if the selection pressures operating on sperm length differ from those acting on the length and number of female ssts. For example, an increase in the length of ssts in females could force sperm to form layers within each tubule. When sperm are layered in an sst it is thought that the last sperm entering the sst will be the first to leave and fertilize the next egg laid (Birkhead & Hunter 1990). Thus a female could conceivably control the paternity of her eggs simply by ensuring that the most desirable male is the last to mate with her before fertilization. Males, however, could counter this tactic by evolving sperm long enough to fill these longer ssts, thereby again excluding sperm from subsequent inseminations. If no agreement between the sexes over the pattern of sperm storage is reached, selection on both sperm size and sst size may escalate into an ‘arms race’ (Dawkins & Krebs 1979). It seems to us that only such a process can explain the large differences in sperm size, even between closely related species.

The number of ssts present in a female might also be a limiting resource for male sperm: in poultry, the majority of sperm in an ejaculate do not get stored in ssts (Allen & Grigg 1957; Howarth 1974; Brillard & Bakst 1990), and a single (artificial) insemination is enough to fill ssts to capacity (McIntyre & Christensen 1983). We have similarly observed that the ssts of passerine birds are often filled (J. V. Briskie & R. Montgomerie, unpublished data), suggesting that ssts may be in short supply, at least in some species. Because the swimming speed of sperm appears to be positively correlated with sperm length (Gomendio & Roldan 1991), selection should favour faster swimming speed when ssts are in short supply. Thus a negative correlation between sperm length and the number of ssts (figure 4b, d) is also expected if competition over storage sites influences sperm length.

Unlike a recent study of sperm size and sperm competition in mammals (Gomendio & Roldan 1991), we did not find a significant correlation between sperm size and mating system in birds. Although we analysed data from only 20 species, the expected pattern was not found either when examining the relation between sperm length and testis size (table 1), or when comparing the average sperm lengths of monogamous and polygynous species. An analysis within a relatively small taxon exhibiting a diversity of mating systems (e.g. Scolopacidae (Pitelka et al. 1974)) might provide a more convincing test. Within different primate taxa,
for example, polyandrous species almost invariably had longer sperm than monandrous species (figure 2b in Gomendio & Roldan 1991) and the pattern was similar across primates and muruid rodents, separately.

The expected correlation between sperm size and mating system might not always obtain even in the face of intense sperm competition, if sperm is stored before fertilization. In many bird species, copulations occur less frequently or cease altogether during the actual period of fertilization (usually from the day before the first egg is laid to the laying of the penultimate egg (Birkhead 1988)). Thus, in those species, sperm competition for access to storage sites (ssts) might be expected to be more important than competition either among sperm for direct access to unfertilized ova or among males for access to females. Given that females in a wide variety of taxa – including some mammals (e.g. bats (Racey 1979)) – store sperm, a general correlation between sperm size and mating system should not always be expected in all animals. Conversely, it will be interesting to see if there is a correlation between sperm length and mating system in those birds in which copulations occur throughout the period of fertilization (see Birkhead et al. 1987), when sperm can gain direct access to unfertilized ova without an intervening period of storage in ssts. Such a correlation would be predicted for all of the reasons enumerated by Gomendio & Roldan (1991).

The results of our study suggest that ssts (both their size and number) are more important than other variables in determining interspecific variation in sperm length in passerine birds. Although the correlations are clear, the mechanisms leading to the relations between sperm length and both the number and length of ssts may be complicated. At the most fundamental level, for example, we need to know how sperm size, sperm layering and sperm precedence vary among species with different mating systems and patterns of copulation.

The absence of the expected correlations between sperm length and social mating systems in birds also indicate that we really know very little about the genetic aspects of mating systems in most bird species, even though the social aspects of mating systems have been well documented (Davies 1991). The correlations between sperm length and both sst length and number that we report here suggest that there may be a level of complexity in interactions at the genetic level that will be as fascinating as the behavioural interactions during mating that have been the focus of so much research to date.

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REFERENCES


Amadon, D. 1943 Bird weights and egg weights. Auk 60, 221-234.


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