

## Male-specific differences in the nuclear and flagellar lengths of spermatozoa from Asian houbara bustards *Chlamydotis macqueenii*

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Ejaculates from individuals of the African houbara bustard, *Chlamydotis undulata undulata*, have been shown to contain up to 40% of spermatozoa with large nuclei, which contain excess DNA. In this work, we have sought to determine whether differences in this trait and flagellar length are also found in the Asian houbara bustard, *Chlamydotis macqueenii*. Measurements of individual sperm features were performed on digital images of aniline/eosin-stained spermatozoa. All statistical analyses were carried out using Minitab version 13.

In samples from the 2001 season, the frequency distributions of sperm nuclear lengths from 9 birds showed normal distributions, distributions that were skewed to the right, and distributions that showed two distinct populations (see Figure 1). There were no significant differences in the proportion of enlarged nuclei in pairs of ejaculates from the same bird ( $\chi^2$ : each  $P > 0.05$ ), however, significant differences in the proportion of enlarged nuclei were observed between birds ( $\chi^2$ :  $P < 0.05$ ). No significant differences in the mean length of nuclei between ejaculates in males were found in 8/9 birds (Two sample t-test:  $P < 0.05$ ), although there was variation between males (ANOVA:  $P < 0.001$ ). Details are shown in Table 1.

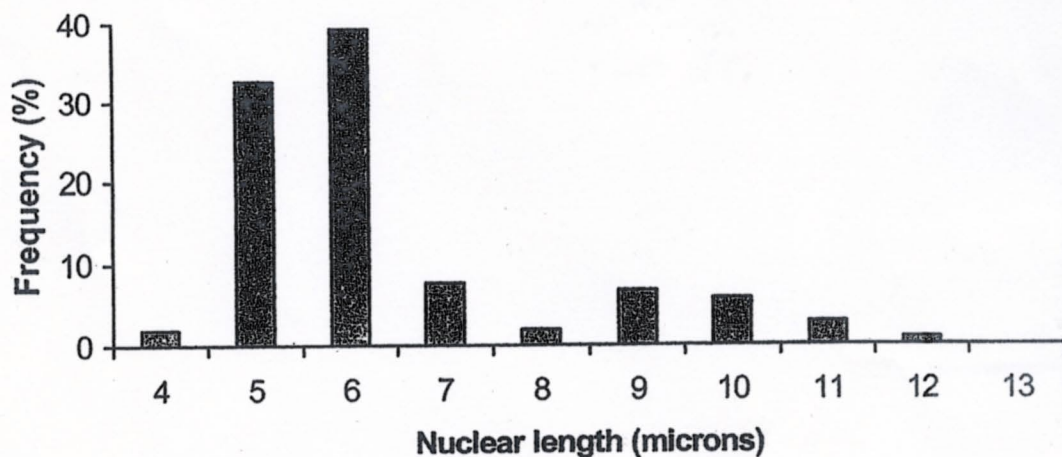


Fig. 1 Frequency distribution of sperm nuclear lengths in bird A

In the 2002 season, the proportions of spermatozoa with large nuclei from birds A, H and C were 14.9%, 8.9% and 5.6%, respectively. The proportion of spermatozoa with large nuclei in samples from birds A and H were not significantly different between breeding seasons ( $\chi^2$ : each  $P > 0.05$ ), but those from bird C showed significant differences between seasons ( $\chi^2$ :  $P < 0.05$ ).

Typically, flagellar lengths were normally distributed in all nine birds studied in 2001 (examples shown in Figure 2). Sperm flagellar lengths in ejaculates from six of nine birds were not significantly different (two sample t-test:  $P > 0.05$ ). However, samples from individual birds showed differences in flagellar lengths (ANOVA:  $P = 0.001$ ; Table 2). The flagellar lengths in samples from birds A and C, but not H, showed some differences between seasons (ANOVA:  $P < 0.05$ ), but the means of the flagellar lengths from all these three birds retained the same ranking between the two seasons.

Table 1 Characteristics of sperm nuclear lengths in two ejaculates from different birds

Bird	Mean $\pm$ SD of main distribution of nuclear length ( $\mu\text{m}$ )	Proportion of sperm outside of normal distribution %	
		Ejaculate A	Ejaculate B
A	6.0 $\pm$ 0.8	5.7 $\pm$ 0.8	16.0
B	4.3 $\pm$ 0.6	4.0 $\pm$ 0.4	0
C	5.6 $\pm$ 0.9	5.4 $\pm$ 0.7	0
D	6.1 $\pm$ 1.2	5.1 $\pm$ 0.7	5.8
E	6.6 $\pm$ 0.8	6.4 $\pm$ 0.9	2.0
F	6.2 $\pm$ 0.6	5.8 $\pm$ 0.6	8.3
G	6.1 $\pm$ 0.5	6.1 $\pm$ 0.5	18.6
H	6.7 $\pm$ 0.7	6.5 $\pm$ 0.8	11.0
I	7.2 $\pm$ 0.8	9.5 $\pm$ 0.9	40.0

The presence of spermatozoa with populations of nuclei that appear to be nearly twice the size of that of the main population (and may be diploid) is now demonstrated in *C. macqueenii*. Furthermore, whilst there are clearly male-specific differences in the proportion of large-nuclei spermatozoa, these have not shown the same ranking between seasons and cannot, therefore, be proven to be a male-specific trait. The physiological effect of such large-nuclei spermatozoa remains unknown, but they are not, in *C. undulata undulata*, associated with abnormal ploidy in breeding males or their offspring. Individual birds produced spermatozoa with different flagellar lengths, but whether or not a longer flagellum is associated with faster sperm motility and increased fertilising potential, remains unknown.

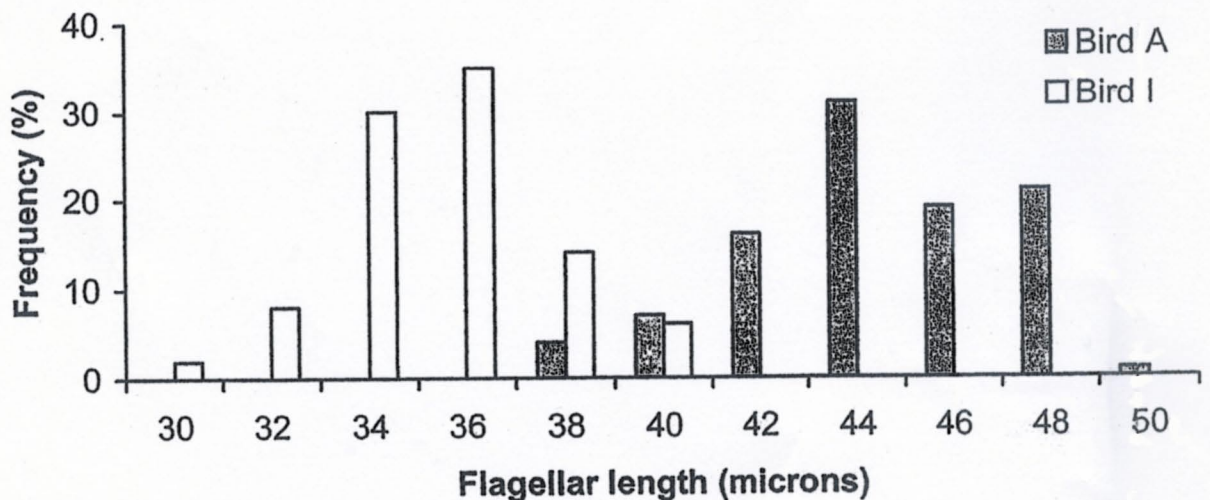


Fig. 2 Frequency distribution of sperm flagellar lengths in bird A and I

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