Why do female adders copulate so frequently?

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MALES of most animal species will enhance their reproductive success if they mate often and with many different partners, whereas promiscuous mating is unlikely to increase a female's reproductive success. Why then is multiple copulation by females so common? Many theoreticians have suggested that multiple copulations might enhance the viability of a female's offspring, either because of inadequate quantities of sperm from the first mating, additional nutrients derived from the seminal fluid or some genetic advantage. Our field studies on Swedish adders provide the first empirical evidence that multiple copulations, with different partners each time, increase offspring viability. This advantage apparently results from more intense sperm competition in the female's reproductive tract, resulting in a higher proportion of her ova being fertilized by genetically superior males.

adders (Vipera berus) are small venomous snakes widely distributed throughout Europe. Like all snakes, they lack male parental care, and female cooperation is required for successful copulation. Our field studies on Swedish adders provide the first empirical evidence that multiple copulations, with different partners each time, increase offspring viability. This advantage apparently results from more intense sperm competition in the female's reproductive tract, resulting in a higher proportion of her ova being fertilized by genetically superior males.

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populations such as that of the Smygehuk adders, because of the high potential for inbreeding depression\textsuperscript{19}. The mating system of adders, whereby a single large male may obtain most of the matings, reduces effective population size and thus amplifies this effect\textsuperscript{20}. The Smygehuk adders offer an ideal opportunity to look for an influence of multiple mating on offspring viability, because genetic bottlenecks and small effective population sizes have resulted in low genetic variability and high proportions of stillborn offspring in most litters (mean = 31.6\% of offspring stillborn, s.d. = 27.4, range = 0–100\%, n = 34 litters).

Multiple matings strongly reduced the proportions of offspring that were dead at birth (correlating proportion of stillborn young against number of matings, $r = -0.40$, $n = 34$, $P < 0.02$; against number of different males mated with, $r = -0.40$, $n = 34$, $P < 0.02$). No other variable that we measured on females or their litters was significantly correlated with the proportion of stillborn young (for example litter size, $r = 0.04$, $n = 34$, $P = 0.25$; mean offspring mass, $r = 0.24$, $n = 30$, $P = 0.20$), and there were no consistent differences in this respect between first and second litters of the same female ($r$-test, 8 d.f., $t_{8} = 0.24$, $P = 0.81$), or among individual females (using a one-factor analysis of variance, with identification number of the female as the factor, $F_{1,6} = 1.18$, $P = 0.42$). The number of times that a female mated also did not differ consistently among individual females between years ($F_{1,6} = 1.24$, $P = 0.39$), or between first and second litters ($t_{8} = 0.79$, $P = 0.45$). Hence the number of matings (or numbers of different mates, which is highly correlated with number of matings; $r = 0.90$, $n = 34$, $P < 0.001$) seems to be the primary determinant of the proportion of viable offspring produced by a female adder.

More detailed analysis shows that offspring viability depends on a female’s choice of mates as well as the number of times she copulates. We looked for a paternal effect on offspring viability by restricting analysis to litters that had been sired by only one or two males, and comparing the proportion of stillborn offspring among fathers. The resulting one-factor analysis of variance (with identification number of the male as the factor) revealed strong differences among the seven males for which data were available ($F_{6,2} = 101.8$, $P < 0.01$), with matings by two of the males producing 100\% inviable offspring, 67\% in two others and 29–33\% stillborn offspring in litters sired by the other three males. Multiple mating results in shared paternity of the offspring.\textsuperscript{12} Our data suggest that the ova of females that mate more frequently are fertilized mostly by the sperm of ‘better’ males. If this were not the case, a higher number of copulations might reduce the variance in proportions of inviable offspring in a litter, but should not affect the mean proportion of inviable neonates. Instead, our data show that the proportion of viable offspring increases with additional matings.

Why should a higher number of matings per litter result in fertilization of a female’s ova by a ‘better’ male? Multiple mating increases the potential for intrauterine competition among sperm from different males. If sperm that are more successful in fertilizing the ova are also more effective in producing viable offspring (as shown in some inbred populations of mammals\textsuperscript{13,14}), then multiple mating should increase the average viability of offspring\textsuperscript{15,16}. Such an effect would normally be difficult to detect because of the problems inherent in assessing offspring quality, and the high proportion of stillbirths in the Smygehuk population overcomes this problem. Nonetheless, the same effect may well be widespread among other populations of adders (and many other types of animals). The enhancement of offspring quality through intrauterine sperm competition offers a plausible selective advantage for the willingness of females of many species to mate frequently, with many partners.

**Retinoid X receptor is an auxiliary protein for thyroid hormone and retinoic acid receptors**

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**THYROID hormones and retinoic acid function through nuclear receptors that belong to the steroid/thyroid-hormone receptor superfamily (reviewed in refs 1–4). Thyroid hormone receptors (TRs) and retinoic acid receptors (RARs) require auxiliary nuclear proteins for efficient DNA binding\textsuperscript{5–10}. Here we report that retinoid X receptors RXR\textsubscript{\alpha} (ref. 11) is one of these nuclear proteins. RXRX\textsubscript{\alpha} interacts both with TRs and with RARs, forming heterodimers in solution that strongly interact with a variety of T3/retinoic acid response elements. Transfection experiments show that RXR\textsubscript{\alpha} can greatly enhance the transcriptional activity of TR and RAR at low retinoic acid concentrations that do not significantly activate RXR alone. Thus, RXR\textsubscript{\alpha} enhances the transcriptional activity of other receptors and its own ligand sensitivity by heterodimer formation. Our studies reveal a new subclass of receptors and a regulatory pathway controlling nuclear receptor activities by heterodimer formation.**

**We investigated the possibility that the auxiliary nuclear proteins might be members of the nuclear receptor proteins, in particular those that bind and activate the same or related response elements. Using a gel retardation assay, we observed that TR\textsubscript{\alpha} DNA binding greatly increases in the presence of RXR\textsubscript{\alpha}. A prominent complex which migrated much more slowly than the monomeric TR\textsubscript{\alpha} complex was observed, whereas TR binding was reduced (Fig. 1a). At the concentrations used, RXR\textsubscript{\alpha} alone did not form visible complexes with the thyroid hormone response element (TRE). By contrast, there was no change of TR binding when mixed with RAR\textsubscript{\alpha} (Fig. 1a) or oestrogen receptor (ER) (Fig. 1b). In addition, when RXR\textsubscript{\alpha} was mixed with oestrogen receptor and labelled oestrogen response element (ERE) (Fig. 1b), no increased binding or slow electrophoretic mobility complex was seen. When the carboxy-terminal variant TR\textsubscript{\alpha}-2 (ref. 12), which is not a transcriptional activator\textsuperscript{11,15}, was used, a low-electrophoretic-mobility complex did not form either (Fig. 1a).**

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