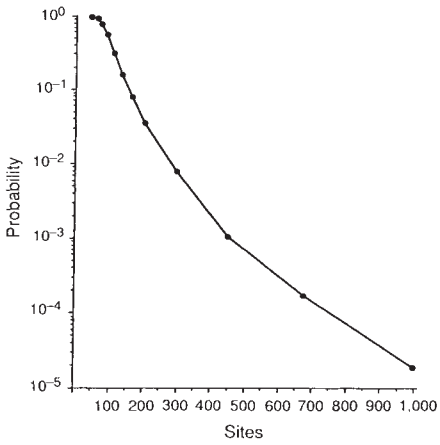

Probability that at least 5 of 10 introns randomly inserted in one gene will match any of the 42 intron positions in another gene, as a function of target sites available for insertion (data from computer simulations).

of chloroplast origin) and the forty-two positions for *gapC* genes. Separate insertions cannot explain this pattern, they argue, and these spliceosomal introns must therefore have been inherited from an ancestral gene. The authors suggest that the introns predate the divergence of eubacteria and eukaryotes, even though spliceosomal introns have been found only in genes in the eukaryotic nucleus[4], and not in the thousands of genes examined in prokaryotes.

Kersanach *et al.*[1] examine the possibility of separate insertions using a model in which introns insert "randomly with a homogeneous probability"[7] at each possible site. The resulting probability of five matching positions, 2.2×10^{-5}, allows them to exclude this model. But a homogeneous insertion probability would not have been expected, since mobile elements typically exhibit heterogeneous probabilities, striking some sites more often than others. Many examples of this principle could be given, the most relevant being the recent demonstrations[8,9] of the mobility of group II self-splicing introns (distant relatives, perhaps, of spliceosomal introns), which included evidence of recurrent insertions into the same site[8], and sequence similarities among different insertion sites[9].

The greater the preferences, the greater the chance that introns inserted into separate copies of a gene will match. The figure shows this effect for an idealized pair of *gap* genes. The chance of obtaining five or more matches goes up dramatically as the number of target sites for insertion goes down. For instance, if introns insert at a 2-nucleotide target site, only 62 (on average) of the 998 possible intron positions (in a 333-codon *gap* gene) would be targets, and five (or more) matching intron positions would not be rare, but quite common ($P > 0.9$). This idealized treatment ignores nucleotide sequence divergence, which must have slowly reduced the tendency for preferred insertion sites (and thus, intron insertions) to coincide. Nevertheless, this is a comparatively minor effect, as the sequences of *gapAB* and *gapC* genes are still quite similar.

Either spliceosomal introns are mobile elements that can insert at matching positions in different copies of a gene, or they are ancient relics lost from nearly all bacterial genes. The difficulty with the latter position lies in explaining why the tiny fraction of bacterial-derived genes that have 'retained' introns (for example *gapAB* genes) is so conspicuously identical with the tiny fraction of bacterial-derived genes that (due to lateral transfers) resides in the nucleus (for example *gapAB* genes). Compared to this riddle, explaining a few matching intron positions is simple: spliceosomal introns, like lightning and, more importantly, like other mobile elements, often strike the same place twice.

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CERFF *ET AL.* REPLY — Logsdon/Palmer and Stoltzfus suggest that the five identical intron positions across chloroplast and cytosolic GAPDH genes (*GapA/B* and *GapC*, respectively) are best explained by parallel insertions at common target sites rather than by common ancestry. But if relatively late targeted intron insertions played a major role in GAPDH gene evolution, one would expect to find coincident intron positions preferably across the related *GapC* genes of plants, animals and fungi, rather than across the highly divergent paralogous gene types *GapA/B* and *GapC*. Precisely the opposite is observed. Only one GAPDH intron (No. 15 in Fig. 1 of ref. 1) fulfils the former criterion, whereas five fulfil the latter. Phrased another way, why does lightning (Stoltzfus), if it strikes twice, strike preferentially at distantly related 'protosplice sites' in the most divergent genes and avoid more closely related ones in genes of more recent common descent? Without invoking complex auxiliary assumptions, the 'introns-late' hypothesis cannot account for this observation in GAPDH genes, while retention of ancient introns and differential intron loss ('introns-early' hypothesis) readily explain the data.

Logsdon and Palmer's rationale in favour of a late lineage-specific acquisition of GAPDH introns is based to a large extent on negative evidence: lack of introns in many GAPDH genes. For example, *Arabidopsis GapC* lacks three introns (Nos 34, 44 and 46 in Fig. 1 of ref. 1) which are present in maize and pea *GapC*: three losses in *Arabidopsis* are more likely than six independent gains at three identical positions in pea and maize. There are other such examples which clearly contradict the *a priori* notion of Logsdon and Palmer that intron gain should be generally more plausible than intron loss. Ironically, Logsdon and Palmer, who support intron mobility[4], also reject categorically the idea of 'intron slippage'[10,11], the occasional displacement of an intron over a short distance of a conserved coding sequence (not to be confused with 'intron sliding', the sliding of a *single* intron junction leading to insertions/deletions[12]) and on the basis of this implicit axiom "once inserted, always immobile" invoke "too tiny" ancestral GAPDH exons as evidence against the exon theory of genes. We maintain that the numerous cases of quasi conservation or clustering of GAPDH introns may be best explained by intron slippage and not by independent intron insertion, which is particularly evident for introns 37 and 38 which are separated by one nucleotide in *GapC* genes of three related basidiomycetes sharing several additional introns at identical positions (see Fig. 1 of ref. 1). We have previously argued[10,11] that both loss and slippage of introns can be rationalized in terms of rare, occasional splicing errors leading to modified pre-mRNAs which can then be re-introduced into the genome via reverse transcription and gene conversion.

Finally, 'introns late' supporters need to address the critical question at hand, how were genes and long contiguous open reading frames assembled in early evolution? If they do not accept the concept of intron-mediated 'exon-shuffling' for primordial gene evolution[6], then they should suggest some plausible alternative hypothesis with testable predictions. In conclusion, the GAPDH gene system is the only current example of ancient gene diversity comprising a sufficiently large dataset to allow direct discrimination at the gene level between the 'introns-early' and the 'introns-late' view and it clearly provides positive evidence in favour of the former. The recent discovery of a 'classic' GAPDH in the archaebacterium *Haloarcula vallismortis*[13] and the characterization of the corresponding operon (unpublished data) indicate that the *GapA(B)/GapC* gene duplication, indeed, occurred

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in the progenote. As information from other genes of similar antiquity becomes available, more clarity should ensue. It is the data, rather than arguments, that are lacking.

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Can female adders multiply?

SIR — Madsen *et al.*[1] conclude from a field study on an adder *(Vipera berus)* population from southern Sweden that most of the females mated multiple times in a season, and that those females that copulate more frequently than others also produce a higher mean number of viable offspring. These findings suggested to the authors that the increase in viability of fertilized eggs is due to sperm competition, in which the 'best' sperm compete for the chance to effect fertilization. But in an adder population from northeastern Italy (Sella Nevea, Carnic Alps, 1,100 m high), we find that only about 18% of the females mated multiple times in a season, and those females copulating multiple times very often do so with the same male[2].

In the reproductive period of 1993, we captured 20 free-living adders (belonging to the population studied by Luiselli[2]) immediately after the end of hibernation (before the start of the mating period), and placed them in a outdoor enclosure (in the adder habitat) to monitor the exact number of copulations of each female. Receptive males from the same population were introduced into the enclosure. Ten female individuals were mated only once (group A), and ten were mated multiple times (from 3 to 8, group B). We used differently sized males, but there were no mean size differences between adders that copulated with female groups A or B. After the end of the experiments, we measured clutch parameters of the two groups of females by using methods as described in refs 3 and 4.

As in the study by Madsen *et al.*[1], we found that the number of copulations was not significantly correlated with litter size or with female fecundity relative to body size (in either case, $r < 0.3$, $P > 0.1$), and that the number of matings by a female did not affect her mean offspring mass, her total mass or her proportional body mass loss during gestation (in all cases, $r < 0.3$, $P > 0.1$). But our data did differ from those of Madsen *et al.*[1] in that multiple matings did not reduce the proportions of offspring that were dead at birth. In fact, the proportion of dead offspring per litter was not significantly different in the two groups of females ($\bar{x} = 12.0 \pm$ [s.d.] 16.52% of female group B versus $14.0 \pm$ 17.66% of female group A: two-tailed $t = -0.19$, d.f. = 18, $P > 0.8$), and the correlation between proportion of stillborn young and number of different males mated with was not significant ($r = 0.53$, ANOVA: mean square = 0.069, $F = 3.17$, $P = 0.1$). Thus, at least in the population we studied, the number of different males mated with does not seem to be the primary determinant of the proportion of viable offspring produced by a female adder. Thus, although the arguments of Madsen *et al.*[1] are interesting, we suspect their results may not apply to all adder populations. On the other hand, it could be claimed that the mating pattern is adaptive in each case, and that female adders mate multiply with different males only when there is a positive benefit from doing so.

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SIR — Females of many animal species mate frequently, with several different males. This 'promiscuous' female behaviour is unexpected from simple Darwinian theory because the number of offspring produced by a female does not increase if she has more sexual partners. In an earlier paper[1], some of us suggested that by this behaviour, females promote sperm competition among males, and their offspring are thereby sired by males with 'better' genes. We found that female adders (*Vipera berus*) that mated with several males produced a higher proportion of viable offspring than did 'monogamous' females[1]. Parker[5] suggested that judgement of this hypothesis should be suspended until further evidence was accumulated. Here we report a study of lizards that strongly supports the earlier hypothesis[1]. Not only does multiple mating of lizards with different partners increase hatching success and lower the incidence of deformities, but it also enhances survivorship of free-living juveniles.

We studied a population of marked, blood-sampled sand lizards (*Lacerta agilis*) 50 km south of Gothenburg on the Swedish west coast[6,7]. Matings were directly observed ($n=32$) or were inferred from the post-copulatory mate guarding ($n=108$) that characteristically follows the 2–4-min-long copulation[7]. In 1989 and 1990, we incubated eggs from the female lizards under identical conditions in the laboratory. Hatchlings were marked by toe-clipping and were blood sampled before being released at random sites at the Asketunnan study area. We used DNA fingerprinting[7] to establish paternity and to assess the degree of genetic variation in the population. After one year we recaptured the survivors to determine whether offspring from multiply-mating females were more likely to survive as free-living juveniles.

Genetic variation in the population was low (mean band sharing among individuals was 66%, range, 63–68, $n=30$). Despite the low genetic variation, we could identify male-specific bands in five broods; four had mixed paternity (our unpublished data). Females mated on average 3.7 times with 1–5 different males (mean, 1.7). The resulting clutches varied in hatching success (mean, 81%; range, 38–100). We recaptured 42 of the 516 released hatchlings, with some clutches being much more highly represented than others (mean, 9.5%; range, 0–43%). As some of us predicted (one-tailed tests), a female's number of sexual partners was: (1) positively correlated with the hatching success of her eggs ($r_s=0.59$, $P=0.0003$, $n=31$); (2) negatively correlated with the proportion of hatched young that exhibited malformations ($r_s=0.33$, $P=0.035$, $n=31$); and (3) positively correlated with the proportion of her offspring that were recaptured after 1 year ($r_s=0.37$, $P=0.020$, $n=31$). This result remained significant when clutch size was controlled for in a partial correlation analysis ($r_s=0.41$, $P=0.014$, $n=31$).

Could these differences in offspring viability be caused by nutrients in the ejaculate, rather than by genetic enhancement of offspring? Probably not. Some females mated more than once with the same male, enabling us to examine the effects of number of copulations independently of the number of partners. Increased copulations with the same male did not increase a female's offspring viability (number of matings versus hatching success: $r_s=-0.19$, $P=0.502$; versus % deformities: $r_s=0.42$, $P=0.136$; versus offspring survival: $r_s=0.41$, $P=0.143$, $n=15$).

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