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SHORT REPORT

Extra-pair paternity in Short-toed and Lesser Short-toed Larks *Calandrella brachydactyla* and *C. rufescens*

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Capsule DNA fingerprinting suggests that extra-pair paternity may be frequent among larks.

Extra-pair paternity is widespread in passerines, even in species traditionally considered to be strictly monogamous (Birkhead & Møller 1998), though it remains unreported or not quantified in some taxa. Larks (Alaudidae), a 'primitive' passerine family with over 100 species, are considered monogamous (Dean *et al*. 1992, Cramp 1998) as most are territorial and many show a semi-colonial nesting behaviour (i.e. the species of the genera *Eremopterix* and *Chersophilus*, and some species of *Melanocorhypha*, *Calandrella*, *Eremophila* and *Spyzocorys*; De Juana *et al*. in press). A few species present a non-aggregated spatial distribution of nests: Skylark *Alauda arvensis*, Crested and Thekla Larks *Galerida cristata* and *G. theklae* (Delius 1965, Cañadas *et al*. 1982, Møller 1985). We report for the first time extra-pair paternity in two species of lark in the semi-colonial species of the family.

The study was carried out in two different locations in Spain, Cabo de Gata (southeast Spain, 50 m asl) and Layna (central Spain, 1100 m asl). Cabo de Gata is a semi-arid area where Lesser Short-toed Lark *Calandrella rufescens*, Thekla Lark and Short-toed Lark *Calandrella brachydactyla* are the main breeding lark species. In Layna, with a Mediterranean continental climate, Skylark, Short-toed Lark and Dupont's Lark *Chersophilus duponti* are predominant.

During 1997, individuals of Lesser Short-toed Lark and Short-toed Lark were caught at the nest during chick feeding using a special trap (Herranz *et al*. 2000). Birds were colour-ringed and recaptured. In 14 and three nests of Lesser Short-toed and Short-toed Lark, respectively, blood samples were obtained from the chicks and adults to carry out parentage analysis. DNA was extracted from blood following a standard phenol-chlorophom protocol (Bruford *et al*. 1998), and multilocus DNA fingerprinting was used to assess the occurrence of extra-pair paternity in the sampled families. Unfortunately, we could only obtain good quality DNA for the analyses from three families (one Lesser Short-toed and two Short-toed Larks).

Around 5–7 µg of DNA of each individual was digested with *Mbo*I, and electrophoresed for 45 h at 70 V through 1% agarose gels in 1 × TBE, running together adults and nestlings from the same nest. DNA was transferred to nylon membranes by Southern blotting overnight and these were hybridized with ^{32}P-labelled Jeffrey's probe 33.6 (Jeffreys *et al*. 1985). For paternity exclusion, we used the number of novel bands (those not shared with either social parent) to decide whether a particular male or female was the genetic parent of a chick (Westneat 1993). In the two cases of extra-pair paternity reported here, only one of the non-maternal bands of the chicks could be assigned to the social father, which we interpret as strong evidence that the individual was not the true genetic father of the chick.

In the Lesser Short-toed Lark nest we were able to analyse, there was only one chick, which shared seven out of 15 bands with its mother but only one with the male feeding the chicks (i.e. seven novel bands). Regarding Short-toed Larks, in one family comprising one chick and the social father, the chick shared 50% (n =16) of its bands with the male, and was considered to be his offspring. However, in the other family,

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comprising male, female and three chicks, two of the chicks shared all their bands but two (i.e. two novel bands out of 11 and 12 respectively) with male and female, and the other shared 42% of its bands with the female but just one out of 12 with the male (i.e. six novel bands).

In summary, although the scarce data available do not allow quantification, we found strong evidence of cuckoldry in these species, since two out of five chicks in two out of three families sampled were extra-pair offspring. A precise estimate of the rate requires further work. Additional observational data suggest that extra-pair relationships or other forms of complex social relationships may occur in Lesser Short-toed Larks. In two nests in which we could not complete paternity analyses, two different males were caught feeding the chicks (n =23), and in one more nest where the male died, a second male continued feeding the chicks. These types of behaviour were not recorded in any of the Short-toed Lark nests (n = 25).

There are no data on the occurrence and extent of extra-pair paternity in larks. However, because many species are semicolonial and male–male encounters and chasing behaviours are common, cuckoldry may be frequent within the family, as suggested by Møller (1991). Our data from these two lark species, along with other unpublished observations on a male Thekla Lark feeding chicks at two different nests simultaneously and a female shifting from one male to another between two consecutive clutches, support this suggestion. To our knowledge this represents the first record of extra-pair paternity in larks, considered to be one of the more interesting and unknown subjects to investigate in larks (Beason 1995).

In larks, parental care and nestling feeding are provided by both sexes. The finding of two males feeding chicks in one nest can be interpreted in terms of shared paternity, but we cannot rule out the possible presence of helpers at the nest, although this behaviour is probably rare in larks, as it has been documented in only one species (Spike-heeled Lark *Chersomanes albofasciata*; Steyn 1988).

Our data, although scarce, suggest that extra-pair paternity is a common feature in at least these two lark species. We hope that our study will stimulate further work on this topic.

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