Use of microsatellite-based paternity assignment to establish where Corn Crake *Crex crex* chicks are at risk from mechanised mowing

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We used microsatellite DNA to assign probable parentage of young Corn Crakes to adult males and females and use these assignments to estimate the distribution of distances between broods of chicks and juveniles and the night-time singing place of the father at the time of initiation of the clutch. Estimated distances for broods of young chicks were in accord with those estimated previously by radio-tracking, but distances were greater for older unfledged independent chicks not studied previously. Our results indicate that modifications of the timing and method of mowing to reduce losses of nests and chicks should be implemented inside an area within about 500 m of the singing places of male Corn Crakes, rather than the 250 m previously considered to be safe.

Keywords: age-related movement change, agri-environment, conservation management, ranging behaviour.

The Corn Crake *Crex crex* is a migratory rail which breeds in tall vegetation in Eurasia. Populations in western Europe, including the UK, declined markedly, co-incident with the introduction of mechanised mowing of grass (Norris 1947, Green 1995, Green *et al.* 1997a), which destroys nests and kills chicks (Norris 1947, Tyler *et al.* 1998). The Corn Crake is red-listed in the UK Birds of Conservation Concern assessment (Eaton *et al.* 2015) because of its decline, but a partial recovery since the 1990s coincided with encouragement to farmers, through payments from conservation bodies and government agri-environment schemes, to delay mowing and to adopt Corn Crake-friendly mowing methods (O'Brien, *et al.* 2006). The latter at least halves the proportion of chicks killed by mowing (Green *et al.* 1997b, Tyler *et al.* 1998). Knowledge of the location of nesting adult female Corn Crakes and their flightless chicks would be useful for targeting these actions, but the only practical way to determine locations of Corn Crakes is to survey singing adult males at night. Radio-tracking of adult male and female Corn Crakes in Scotland showed that both sexes were often sequentially polygamous and formed short-term pair bonds during which the female laid eggs in a nest close to (range 45–160 m; mean 101 m; N = 9) the night-time singing place of the male (Tyler & Green 1996). Radio-tagged females with chicks (N = 32) used a small brood-rearing area (mean extent of 3.2 ha) around the nest site during the period of dependence (12-18 days) (Tyler 1996), but less is known of the movements of chicks between independence and fledging at about 45 days of age. Most females produced two broods of young per year and incubated their eggs and reared their young hidden in tall vegetation (Green *et al.* 1997b). Females, nests and young cannot be surveyed by any known method. The distribution of nests and young might therefore differ from that of males.

In this paper, we use paternity assignments of captured chicks and juveniles, based upon DNA sampling of the young and adult males, to estimate distances between unfledged chicks at risk from mowing and the singing place of their father. We assess the implications of these results for the conservation management of Corn Crake breeding areas.

METHODS

Surveying, catching and sampling singing adult male Corn Crakes

We studied a re-introduced Corn Crake population at the Nene Washes (52.58°N, 0.07°W) in Cambridgeshire, England, UK, centred on a nature reserve owned and managed by the Royal Society for the Protection of Birds (RSPB). Night-time surveys of singing male Corn Crakes were conducted in May–July of 2013, 2014 and 2015, commencing when Corn Crakes arrived in the breeding area from their spring migration (Table 1). As many of the males as possible were captured at night by luring them into mistnets using a broadcast recording of conspecific song. Each bird was marked individually with a numbered BTO metal ring, or a previously applied ring was read, and a sample of buccal epithelial cells obtained using a cotton swab. Appendix S1 gives further details of the study area and methods.

Drive catching and sampling of adults, chicks and juveniles

Corn Crake adults, chicks and juveniles were captured by driving them into funnel traps in July-August. For each drive, an approximately rectangular area of 1.2 – 4.7 ha of tall grass and herbage was enclosed by a combination of fences of netting and existing barriers, such as water-filled ditches. Corn Crakes within it were driven towards a line of traps linked by drift fences set at one end of the drive area. It was not possible to conduct drive catches over the whole study area, but drive areas were widely spread. Further details of the method are given in Appendix S1.

Birds were captured in the funnel traps, except in one instance when downy chicks estimated to be seven days old were seen during a drive. One chick from this brood was captured by hand near where it was first detected, to reduce disturbance. The assumed location of this brood before disturbance was the actual capture location because chicks of this age move slowly in response to disturbance (Tyler *et al.* 1998), but in all other cases the brood location before disturbance occurred was taken to be the centre of the drive area. Although the locations of broods before the disturbance caused by the drive would have been distributed within the drive area, we took its centre to be a reasonable approximation of the mean of possible undisturbed positions when calculating the distance of chick locations to the singing place of their father. We assessed the sensitivity of our conclusions about chick-father distances to this assumption by measuring the shortest and longest distances between any part of the drive area in which a chick was captured and the father's singing place.

The age of captured young was estimated from measurements, using methods described in Appendix S1. The date of laying of the first egg of the clutch from which they hatched was estimated using the mean age of the brood and assuming 26 days between first egg and hatching date. Eight days is the laying period of a typical clutch and 18 days is the usual incubation period (Green *et al.* 1997b).

Buccal swab samples were collected as for singing males. Genomic DNA was extracted and genotyped for 15 microsatellite loci. Parentage assignment was performed from data for adults and young using methods described in Appendix S1.

RESULTS

In each study year, most (71–95%) of the singing male Corn Crakes present were captured and sampled (Table 1). Seventeen of the 43 males were captured more than once during the same breeding season to read the ring number and check their identity. Although most males were recorded as singing within a few hectares throughout the breeding season, some individuals moved up to 1.2 km. Movements exceeding 200 m were detected by recapture for 11 males (26%; Table 1). Microsatellite genotypes were obtained for all 43 of the sampled adult males and for five adult females captured during drives (Table 1).

Paternity was assigned to sampled fathers with a probability ≥ 0.80 for 16 chicks and six juveniles, which were assigned to 14 broods based on their estimated hatching dates (Table 2). Ten sampled adult males were assigned as fathers of captured young. Four of the fathers were each assigned two broods in the same breeding season (Table 2). In three cases, the two broods with the same father had different mothers (broods 1 and 2, 3 and 4, 9 and 10) and in one case the mother was the same for both broods (broods 6 and 7). The two broods with the same mother were captured on the same drive and had first-egg dates which differed by 34 days. Of the three pairs of broods with the same father, but different mothers, the first comprised two fledged juveniles captured on the same drive and the others were captured 1153 m and 168 m apart with first-egg dates 13 and 33 days apart. The locations of broods in relation to all of the recorded singing places of their assigned sires are mapped in Appendix S2.

Broods of chicks up to 20 days old, which would mostly still be dependent on the mother, tended to be close (median 78 m; range 4–151 m) to the singing location of the father, but older unfledged chicks, which would all be independent, were further away (median 261 m, range 149–601 m: Mann-Whitney *U*-test; $U_{3,7}$ = 1, two-tailed P = 0.034; Fig. 1). However, there was no significant correlation overall between the distance from the father's singing place and chick age for unfledged chicks (Spearman's coefficient r_S = 0.225, one-tailed P = 0.266; N = 10). Distances of fledged juveniles from their father's singing location were similar to those of chicks older than 20 days (median 180 m; range 120–823 m; $U_{7,8}$ = 21, two-tailed P = 0.266). The mean distance of all unfledged chicks from the father's singing place was 243 m (se ± 55 m) and the mean distance for fledged juveniles was 298 m (se ± 83 m).

We assessed the sensitivity of our conclusions about unfledged chick–father and juvenile–father distances to the uncertainty about where undisturbed chicks were located before drives began by using the closest and furthest possible locations of the brood, relative to the father's singing place, before it was disturbed by the capture process, instead of assuming that the undisturbed brood was at the centre of the drive area. As expected, the distances obtained from these extreme alternative assumptions were smaller and larger respectively than those obtained using the drive centres, but the results remained broadly similar. If we assumed that an unfledged chick was as close as it could possibly have been to its father, whilst being within the drive area, the mean distance was 163 m (range 0–451 m) and two of the ten observations still exceeded the threshold distance of 250 m previously considered to be safe (O'Brien *et al.* 2016). If it was assumed that an unfledged chick was as far as it could possibly be from its father, the mean distance was 356 m (range 78–724 m) and eight of the ten observations exceeded the 250 m threshold distance. For juveniles, the equivalent mean distances for the closest possible and furthest possible alternative assumptions were 170 m (range 0–711 m) and 447 m (range 278–952 m) respectively.

For four broods, the father assigned to an unfledged brood was the male singing, at around the time of laying of the first egg, closer to the brood's first capture location than any other sampled male; for three broods the father was the second closest male; and, for one brood, it was the third closest male (Table 2). We refer to this relative ranking of the father, relative to other sampled males, as his distance rank. For the fathers of six young birds first captured as juveniles, the distance ranks were 1, 1, 2, 3, 5 and 6 (Table 2). The first location of every brood was much closer to the singing location nearest in time to the first egg dates of the male assigned as its father than the mean distance from the brood location of the singing places closest to that date of all the other sampled males in that year (Table 2). This tendency of broods to be closer to the singing location of the father, than the mean for other sampled males that were not the father, was highly significant (Wilcoxon matched-pairs signed ranks test, one-tailed $P < 0.005$).

Maternity was assigned to sampled mothers with a probability ≥ 0.80 for 18 chicks and three juveniles, which were assigned to seven broods based on their estimated hatching dates. All five sampled adult females were assigned as mothers. Two of the sampled females had two sampled broods in the same breeding season; both broods of one female were sired by the same male with first-egg date 34 days apart, and those of the other female were sired by two different males with first-egg dates 31 days apart.

DISCUSSION

Our results from DNA-based parentage assignment are consistent with those obtained from radio-tracking studies in finding evidence of some males fathering young with more than one female and of young with the same mother from two broods with hatching dates separated by approximately the expected time interval between first and second clutches. We also found that broods of chicks up to 20 days old were within 151 m of the singing location of the father at around the time of the first-egg date of the clutch, which is as expected from the radio-tracking determinations of locations of nests and dependent broods. However, independent unfledged chicks older than 20 days were located at least 149 m, and up to 601 m, from the singing place of their father, and fledged juveniles were up to 823 m away. Our findings were not affected by displacement or disturbance caused by mowing because no mowing had occurred within our study area at the time of drive catching. Guided by the radio-tracking results, the Corncrake Initiative, a conservation project operated by the RSPB, offered payments to farmers for voluntary adoption of delayed and Corn Crake-friendly mowing within 250 m of locations of singing males (O'Brien *et al.* 2006), but our study indicates that 40% of locations of all unfledged chicks were further away than this threshold distance, beyond which unmodified mowing has previously been considered safe. We propose that delayed mowing and Corn Crake-friendly mowing should therefore be deployed up to about 500 m from the singing places of adult males. This increase in distance from the previous recommendation of 250 m is intended to reduce the risk that flightless chicks independent of the mother are killed by mowing. Our results support previous finding that modifying mowing dates and methods within 250 m of male singing places is sufficient to reduce the risk that nests and dependent chicks are destroyed. Protection of fledged juvenile Corn Crakes from mowing is less important because they can escape by flying and are rarely killed by mowing (Green *et al.* 1997b).

There are several potential sources of uncertainty in our estimates of brood–father distance and we assess the importance of these in Appendix S3. The largest source probably arises from our assumption that the unknown undisturbed locations of captured chicks were the centres of drive areas. We tested the robustness of our conclusions to this assumption by making extreme alternative assumptions about where young had been located within the drive areas before disturbance. Even when we assumed that every chick was as near as it could possibly have been to its father's singing location, one-fifth of unfledged chick locations were still more than 250 m away. We therefore suggest that the area within which mowing is considered to be safe for Corn Crake nests and unfledged chicks should be extended from 250 m to 500 m and that methods for the targeting of the location of agri-environment delivery within core areas for the species should adopt this rule.

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SUPPORTING INFORMATION

Appendix S1. Supplementary Methods.

Appendix S2. Maps of all recorded singing locations attributed to individual male Corn Crakes assigned as fathers of captured young.

Appendix S3. Assessment of the potential effects of uncertainty and failure of assumptions on the conclusions of the study.

LEGENDS TO FIGURES

Figure 1. Distances (m) between locations of Corn Crake broods captured as chicks (open circles) and as fully-grown juveniles (filled circles) and the singing location of their father on the date closest in time to the first-egg date of the clutch from which the brood hatched. Distances are plotted against the estimated age of the chicks or juveniles in days. Lines between symbols connect repeat observations of young from the same brood. The filled square and the vertical line through it show the mean and range respectively of the distance of nests of radio-tagged female Corn Crakes from the singing place of the male with which they mated (from Tyler & Green 1996).

Table 1. Surveys and captures of singing male Corn Crakes and drive catching of adults, chicks and juveniles at the Nene Washes in 2013–2015.

Year	2013	2014	2015
Adult male surveys and captures			
Survey period	15 May - 18 July	30 April - 19 July	30 April - 9 July
Survey nights	27	26	24
Singing records	48	174	106
No. singing males	7	22	21
Largest count on 1 night	6	16	10
Date of largest count	26 May	18 June	25 May
Capture events	7	29	27
No. males captured	5	21	17
No. males captured twice or more	2	7	8
No. males moving > 200 m	2	4	5
Maximum movement (km)	1.2	1.0	0.5
Drive captures of adults, chicks and juveniles			
Drive period	1 August - 11 August	23 July - 21 August	26 July - 18 August
No. drives	7	18	8
No. chicks captured	18	8	1
No. juveniles captured	6	4	2
No. adult males captured	1	4	0
No. adult females captured	3	2	0

Table 2. Captures and recaptures of 14 broods of Corn Crake chicks and juveniles with fathers identified by microsatellite-based paternity assignment with probability ≥0.80. Brood numbers underlined have an assignment probability ≥0.90. Broods marked with asterisks in the age at capture and first-egg date columns were first captured as juveniles with fully-grown primary feathers, so their age estimate is approximate. The mean distance of the brood from non-fathers is the mean of distances from the capture location of the brood to the singing places, on the date nearest to the first-egg date of the clutch, of the DNA-sampled male Corn Crakes that were not the father of the brood. The distance rank is the rank distance from the brood location to the singing place of the father relative to that of the other sampled males in that year (i.e. 2/21 means that the father's singing location at the date closest in time to the brood's first-egg date was the second closest to the brood location of the 21 males sampled). These two measures are only shown for the first capture of each brood. The first-egg dates are given as days elapsed after 31 December of the previous year.

Year	Brood code	Brood members	Brood members captured	Father	Brood age at capture (days)	First-egg date	Distance of brood from father's singing place (m)	Mean distance from non-father's singing places (m)	Distance rank of father's place
2013	1	EY11035	EY11035	EG59372	50*	138*	148	1505	1/5
2013	2	EY11036	EY11036	EG59372	50*	138*	148	1505	1/5
2013	3	EY11034	EY11034	EG59373	31	155	261	1632	1/5
2013	4	EY11041, 42, 45, 64	EY11041, 42, 45	EG59373	20	168	4	1068	1/5
2013	4	EY11041, 42, 45, 64	EY11045	EG59373	28	168	296	-	-
2013	4	EY11041, 42, 45, 64	EY11064	EG59373	28	168	601	-	-
2014	<u>5</u>	EY11304	EY11304	EY11058	50*	130*	201	1938	2/21
2014	<u>6</u>	EY11301, 02, 03	EY11301, 02	EY11114	41	137	149	1858	1/21
2014	<u>6</u>	EY11301, 02, 03	EY11303	EY11114	43	137	312	-	-
2014	<u>7</u>	S102	S102	EY11114	7	171	78	1868	1/21
2014	8	EY11287	EY11287	EY11152	50*	148*	823	1848	6/21
2014	<u>9</u>	EY11263, 64, 86	EY11263, 64	DE32711	38	151	244	1829	2/21
2014	<u>9</u>	EY11263, 64, 86	EY11286	DE32711	43	151	142	-	-
2014	<u>9</u>	EY11263, 64, 86	EY11263	DE32711	47	151	180	-	-
2014	<u>10</u>	EY11289, 90	EY11289, 90	DE32711	14	184	151	1929	2/21
2014	11	EY11285	EY11285	EY11034	22	171	429	1834	2/21
2015	<u>12</u>	EY11445	EY11445	EY11381	50*	131*	607	1318	5/17
2015	<u>13</u>	EY11455	EY11455	EY11110	50*	136*	120	1090	3/17
2015	<u>14</u>	EY11444	EY11444	EY11251	33	148	212	1484	3/17

Figure 1. Distances (m) between locations of corncrake broods captured as chicks (open circles) and as fully-grown juveniles (filled circles) and the singing location of their father on the date closest in time to the first-egg date of the clutch from which the brood hatched. Distances are plotted against the estimated age of the chicks or juveniles in days. Lines between symbols connect repeat observations of young from the same brood. The filled square and the vertical line through it show the mean and range respectively of the distance of nests of radio-tagged female corncrakes from the singing place of the male with which they mated from Tyler & Green (1996).